UNIVERSITY OF IOWA

ZOOLOGICAL LABORATORY

THE STATE UNIVERSITY OF IOWA

VOLUME I

COLLECTED PAPERS

1927 - 1928

Edited by F. A. STROMSTEN

IOWA CITY, IOWA
U. S. A.

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Temperature Coefficients for Development in Cladocerans.

L. A. BROWN. (Introduced by W. W. Swingle.)

From the Zoological Laboratory, Harvard University.

In an earlier paper[1] it was shown that the temperature characteristics for the duration of an adult instar in several species of cladocerans were of the same order of magnitude as those found for development in other arthropods, but that certain specific differences were still markedly manifest. The temperature characteristics for the different temperature ranges and also the position of the critical temperatures or "breaks" in the *log rate* against *reciprocal absolute temperature* graphs were accorded a natural interpretation in terms of the geographical distribution and seasonal rhythms of the species studied. The present paper presents data on the temperature coefficients for the complete parthenogenetic generation cycle of several common species of cladocerans.

The literature on the biological application of van't Hoff's rule has been summarized by Kanitz[2] and Przibram.[3] Most of the papers dealing with this subject have been concerned with the relative magnitudes of the temperature coefficients and a subsequent classifying of vital activities into those controlled by physical forces and those controlled by purely chemical reactions. The comparison of several species of cladocerans reared in the laboratory for many generations under similar environmental conditions should reveal specific differences, should such be present, and might be taken to suggest a means for the physiological comparison of related forms. In addition to the ease of propagation in the laboratory, cladocerans are well adapted for such a study as their parthogenetic reproduction insures material that is genetically uniform, and their development by instars provides stages which are morphologically well defined.

Six species of cladocerans were used and one of these, *Daphnia*

TABLE I.

°C.	Moina macrocopa		Pseudosida bidentata		Simocephalus, all species		Daphnia pulex (type)		Daphnia longispina		Daphnia magna		Daphnia pulex (984)	
	time in hours	Q_{10}	time in hours	Q_{10}	time in hours	Q_{10}	time in hours	Q_{10}	time in hours	Q_{10}	time in hours	Q_{10}	time in hours	Q_{10}
13	296.8		517.6		381.0		309.7		284.5					
		3.96		2.90		2.36		2.64		1.82				
20	114.2		246.0		208.5		156.8		187.0		203.1		170.3	
		2.39		2.03		1.81		1.70		1.48		1.31		1.19
25	66.9		134.3		129.1		100.8		138.0		146.0		120.4	
		1.76		1.63		1.15								
30	49.5		121.2		115.0		92.1		126.0		155.0		143.2	
35	38.0		82.4		112.5		Will not live and reproduce.							

pulex, included two varieties, the typical form and a thelytokous form[4] which is probably hexaploid in constitution.[5] The following table gives the time in hours from the beginning of the first young instar until the end of the first adult instar, when the females produce their first brood of young, for each of several temperatures and the Q_{10} for the temperature intervals 13° to 20°, 20° to 30°, and 25° to 35° C.

On comparing the Q_{10} of the different species for the temperature interval 20 to 30°, decided differences are apparent. Two of the species show a value of the temperature coefficient which is above 2.00; two with a value near 1.75; one with a value near 1.50; and two with a value near 1.25. The reality of these differences indicated by the temperature coefficient for development is emphasized by comparing the lethal temperatures of the adults of these same species. The lethal temperatures, determined arbitrarily by suddenly immersing the animals in water of the desired temperature for one minute, are: *M. macrocopa* 48°, *P. bidentata* 48°, *Simocephalus* species 43°, *D. pulex* typical 44°, *D. longispina* 42°, *D. magna* 41°, and *D. pulex* "984" 41°. The species are seen to fall approximately into the same groups as determined by the temperature coefficient for development of the younger animals.

[1] Brown, L. A., *J. Gen. Physiol.*, 1926-27, x, 111.

[2] Kanitz, A., *Temperatur und Lebensvorgänge*, 1915, Berlin.

[3] Przibram, H., *Temperatur und Temperatoren*, 1923, Leipiz und Wein.

[4] Banta, A. M., *Z. Indukt. abstammungs u. vererbungl.* 1925, xl, 28.

[5] Schrader, F., *Z. Indukt. abstammungs u. vererbungl.*, 1925, xl, 1.

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3754

Sulfate Retention in Dogs Following Bilateral Adrenal Extirpation.

W. W. SWINGLE and W. F. WENNER.

From the Zoological Laboratory, State University of Iowa.

The results of previous work by the senior writer[1] and his co-workers[2] on the functional significance of the suprarenal cortex, indicated that the cause of death in double operated cats and dogs is probably acid intoxication, due to retention of acids formed in the course of normal metabolism. The acidosis is apparently not due to the formation of abnormal acids within the organism, but due to defective elimination of non-volatile acids.

Study of the acid-base equilibrium of normal and bilaterally adrenalectomized cats showed quite clearly that the animals with marked symptoms of adrenal insufficiency were suffering from an uncompensated non-volatile acidosis. According to the data available, the acidosis was attributed to an increase in phosphoric and undetermined organic acids. However, it was evident that the marked fall in bicarbonate, observed in our double operated animals, was not occasioned to any considerable extent by the rise in inorganic phosphorus, which although marked, never exceeded two millimols, whereas the carbon-dioxide fell from 6 to 10 millimols. We stated that "increase in organic acid (which in our data included the sulfate ion) is the one change of sufficient magnitude to be responsible for the bicarbonate fall. Further efforts on our part to determine the nature of the organic acid or acids ended in failure, hence it was decided to test for sulfate retention, to see if the sulfate ion could be responsible for the observed changes.

Dogs were used as experimental animals, and only those animals surviving 4 to 6 days or longer, following the second operation, were employed for blood tests.* The results obtained are of interest.

Potter, George Edwin

1927. Respiratory function of the swim bladder in Lepidosteus

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RESPIRATORY FUNCTION OF THE SWIM BLADDER IN LEPIDOSTEUS

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THREE FIGURES

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INTRODUCTION

The particular problem with which we are concerned here has to do with the respiratory function of the swim bladder or air bladder in one of the ganoids, Lepidosteus. The respiratory function of the swim bladder is believed to be important in a few species only. From anatomical data alone, several workers have declared the swim bladder to be a respiratory organ in the following genera: Arapaima, Polypterus, Lepidosteus, Amia, Ceratodus, Protopterus, and Lepidosiren. Indeed, it has been observed that a number of these apparently inhale atmospheric air; still there exists no proof, so far as the writer knows, that this respired air is taken into the swim bladder as would be necessary if the organ is to be considered one of respiration. It is known that a number of fish use such parts as the mouth, stomach, intestines,

and branchial chamber as means of respiring atmospheric air. The existing hypothesis that the swim bladder is a respiratory organ is based upon the following facts: *a*) that it has a cellular, spongy appearance internally(10); *b*) that blood is distributed to a rich capillary network in its walls, then collected and carried directly to the heart(11, 23); *c*) that it has a connection with the pharynx by the ductus pneumaticus and glottis(24, 33), and *d*) that apparently the animal gulps air into the mouth(1, 23, 32, 33).

HISTORICAL

The swim bladder is credited with performing a great number of functions in fishes generally. Some of the principal ones attributed to it are: 1) hydrostatic activity, 2) sound production, 3) accessory audition, and, 4) respiration. Hence the rôle that it plays in the life of fishes has attracted the attention of investigators for a long time. Aristotle believed that the organ functioned chiefly in the production of sounds. Borelli[1] (1680) attributed to the swim bladder a hydrostatic function. Delaroche[2] (1809) was among the first to show that an exchange of gas probably occurred between the swim bladder and the blood. However, he did not consider the former an organ of respiration, for the reason that his study was made primarily with physoclistous forms. Van der Hoeven ('41) described the internal surface of the walls of the swim bladder of Lepidosteus as a cellular structure with ridges and pockets. Johannes Müller ('42), meeting with the 'cellular' swim bladders of Lepidosiren, Erythrinus, and others, reduced the question of the respiratory function to a more definite form and answered it to the effect that the 'swim bladder' in Lepidosiren was to be considered a respiratory organ, because it receives 'dark blood' from, and returns 'light blood' to, the heart. But he pointed out that this was not the case in Polypterus, whose swim bladder receives blood

[1] Borelli, G. A. 1680. De motu animalium.

[2] Delaroche, F. 1809 Observations sur la vessie aerienne des Poissons. Annales du Mus. d'Hist. Nat., T. 14, p. 184. Paris.

from the fourth branchial vein and returns it to the liver, or in Erythrinus, where it receives blood from the arteries of the body and returns the blood to the veins of the body. Müller's distinction here is obviously founded upon relations and conditions as they are known in the higher vertebrates.

Hyrtl ('52) studied and described the capillary network of blood vessels which lines the swim bladder of Lepidosteus. He clings to the idea, which was brought out by Müller, that the swim bladder receives only red (arterial) blood and returns only black (venous) blood to the body venous system, hence concluding that its function is not respiratory. Quekett ('44) mentions the close resemblance of the branching blood vessels in the wall of the swim bladder of Lepidosteus to those in the lungs of certain reptiles and suggests a possible analogy.

Poey ('56, '58) made observations upon Lepidosteus and concluded that it has some sort of 'pulmonary respiration.' But he had no direct evidence to show which organ carried on the respiration. Agassiz ('58) called attention to the fact that Lepidosteus is 'remarkable in the large quantity of air which escapes from its mouth,' but he was unable to determine the source of the air.

Wilder ('75, '77) observed the respiratory actions of Amia and Lepidosteus, and concluded that the former and possibly the latter inhaled as well as exhaled air when it came to the surface of the water to 'break,' as the action is called. He also suggested that possibly this air was forced back through the ductus pneumaticus to the swim bladder. Apparently the theory, that certain of the ganoids make use of the swim bladder as a supplementary respiratory organ, has arisen from these observations.

Morris ('85, '92) brought together all of these observations, along with similar ones on some of the dipnoan fishes, to formulate the theory of the origin of the air-breathing land vertebrates. He also referred to the place of these animals in geological history, and finally in his second paper he outlined the origin and development of lungs.

Moreau ('76) did extensive research on the gaseous content of the swim bladder of several European fishes. He showed that there is a constant exchange between the gas in the swim bladder and the gas in the blood

Mark ('90) reports three experiments which he performed in an attempt to analyze the respired air of Lepidosteus osseus. He had only six small animals, and he desired to keep them all alive. His original method for collecting the gas was by the use of an inverted funnel over a jar of water containing the animals. He used this method in one of the experiments only, and then modified it in the others by using an inverted dish instead of the funnel. After allowing the animals to respire beneath the dish for two or more hours he attempted to withdraw the gas from the space between the water and the inverted dish. It is quite probable that all of his samples of gas were contaminated with atmospheric air. The results of these experiments showed that the animals could remove some of the oxygen from the air, but that there was little or no carbon dioxide released here. In two cases he found no carbon dioxide in the gas and in the third he found 1.7 per cent. No attempt was made to take air directly from the swim bladder of the fish. His conclusion is, that Lepidosteus uses the swim bladder as a means of securing additional oxygen, and presumably the gills accomplish most of the elimination of carbon dioxide. The water in which fishes live may be incapable at times of furnishing the necessary amount of oxygen, but sufficiently serviceable as a means of removing carbon dioxide through the gills. And from this he draws another conclusion, that in the transfer of the respiratory function from the gills to the prospective lungs, the oxygenating function was the one first to be transferred. As long as the animal lived in water, the gills were, under all ordinary circumstances, the customary channel for elimination of carbon dioxide; and that, finally, during the passage from water to land, this function was also imposed on the vesicular organ of the intestinal tract, which thus became a lung in the fullest sense of the word. Kerr ('08) pointed out

the possible phylogenetic development of the lungs from the swim bladder. Barrell ('16) went still further and related the Silurian-Devonian climate to this idea of the rise of air-breathing vertebrates, showing the probable influences.

Sorensen ('95) gives a good criticism and interpretation of the important work which has been published on the subject. He points out the relationships of the 'cellular' structure and the vascularization to a possible respiratory function, but indicates that it has never been proved.

Tower ('02) gives a very good account of analyses of gases from the swim bladders of normal and asphyxiated squeteague, a fish with a closed swim bladder. Upon comparing results for the two above conditions, he found more oxygen present in the asphyxiated than in the normal animal, and just the opposite for the carbon dioxide. This is interesting, but is an entirely different condition from that to be shown for Lepidosteus.

Hall ('22) shows that the swim bladder may act as a reservoir on which the blood may draw for oxygen in times of need, enabling even physoclistous fish like perch to go into water of low oxygen content for a short time. In such cases the oxygen gets into the swim bladder by previous absorption from the blood.

MATERIALS AND METHODS

The animals used in these experiments were, for the most part, Lepidosteus osseus (long-nosed gar-pike) and to some extent L. platystomus (short-nosed gar-pike). The individuals selected for the tests ranged from 20 to 30 inches in length. When brought to the laboratory they were placed in large live tanks through which a small stream of fresh water ran continually. Most of the experiments were performed with animals which had been in the tanks only a short time. However, some of the fish were kept in the tanks for months at a time with no apparent discomfort to them. They feed quite well upon small pieces of beef liver. L. platystomus feeds better than the long-nose, L. osseus, and is more hardy for long stays in the live tank.

The general anatomy of the swim bladder and related parts was studied both grossly and microscopically. Several serial sections were made of portions of the bladder in the study of its structure and certain aspects were sketched. Doubly injected specimens were used in the study of the vascular anatomy.

The writer has described an experiment in a previous paper(25) which would indicate definitely that these fish have means of supplementary respiration. The method used was to place a live gar-pike in a vessel containing 5 gallons of water that tested 8 cc. of oxygen per liter (Winkler method). The vessel was covered with a screen to keep the animal from escaping, but there was air space left between the screen and the surface of the water. At the end of twenty-four hours, the water was again tested for oxygen. This test was repeated upon a large number of animals. Following this, experiments were carried on which were similar to the above, except that the screen was lowered to beneath the surface of the water. Control experiments were conducted with a similar set-up, except that a current of oxygenated water was allowed to run through the vessel continually.

In showing that the swim bladder is the organ used for supplementary respiration, paraffin with a melting-point of about 40°C. was melted and injected into the swim bladder by way of the glottis with a large pipette and bulb, subsequently hardening to form a paraffin plug in the anterior end of the bladder. In most cases the swim bladder was not entirely filled for fear the paraffin plug might interfere with the action of other organs of the body. However, a few were entirely filled and it seemed to bother only one such animal. It was found that the smaller amount of paraffin served just as well. The animals were tied, dorsal side down, in a narrow wooden trough, and the mouth tied open for making the paraffin injections. They were then subjected to normal and foul water, and reactions noted. A double control was run in each case: 1) a normal animal being kept in the same container of foul water during the experiment, and, 2) other

injected animals lived in normal water continually during the experiments.

At the end of the experiment the animal used was autopsied to make sure that the paraffin plug was perfect, so that air could not be drawn in.

In the analyses of the gases from the swim bladder, the regular Haldane ('20) gas analysis apparatus(7) was used. The samples tested were drawn from the swim bladder by way of the glottis and ductus pneumaticus with a sampling tube of 25-cc. capacity. This sampling tube is a modification of one used by Hall ('24) in his work with gases of the swim bladder. The samples were introduced into the gas-analysis apparatus on the same day and analyzed in the usual way. Samples of gas from the bladders of fish under various conditions were analyzed.

The apparatus used in showing the rate and extent of the respiratory exchange carried on by the swim bladder of the fish was as follows: a 9-liter glass cylinder, 30 inches tall, fitted with a water-tight lid through which were two tubes, one an outlet and one an intake. The intake tube was connected up with one potassium-hydroxide and two pyrogallol bottles, through which the air was drawn into the cylinder to take place of air removed for samples. The animal was placed in this respiration chamber with about 5 liters of water and enough paraffin oil to make a complete film over the surface of the water. The purpose of the oil was to check as much diffusion between the gas above the water and that in it, as possible. The cylinder was always placed in a horizontal position. In most of the experiments the animals were kept in the chamber for six to eight hours and samples of the gas were drawn at definite intervals to be tested for oxygen and carbon-dioxide content with the Haldane apparatus.

The above-described air-tight cylinder was again used in obtaining the average gram-hour rate of consumption of oxygen by the fish. Instead of leaving an air space above the water, the cylinder was entirely filled with water of known oxygen content so as to exclude all free air. The animal was

placed in the vessel and samples of the water were drawn at definite intervals to be tested.[3] These samples were taken for only two hours in succession, thus avoiding the lowering of the oxygen content to a point where the fish could not pass the water through the branchial chamber fast enough to get sufficient oxygen for normal respiration by gills. Knowing the amount of oxygen consumed in an hour and the weight of the animal, it was possible to compute the amount of oxygen consumed by each gram of the fish's weight per hour. Then, by measuring the capacity of the swim bladder in cubic centimeters and counting the number of inhalations per unit of time, one could show by comparison with the amount of oxygen consumed by the fish whether or not it would be possible for the swim bladder to supply sufficient air for the needs of the body.

ANATOMY OF THE SWIM BLADDER

It is necessary to have in mind several anatomical features of the swim bladder of Lepidosteus in order to understand the significance of the experiments which will be described later in the paper. This organ is situated dorsal to the coelom, between the alimentary canal and the vertebral column. In the adult fish it is just slightly to the right of the median position in the body. Embryologically, the swim bladder arises as a diverticulum from the dorsal side of the alimentary canal. The primitive connection, ductus pneumaticus, is retained permanently as a tube leading from the anterior end of the swim bladder to the slit-like glottis which has a dorsoposterior position in the pharynx (fig. 1).

The internal structure resembles somewhat the air chambers of a primitive lung and has been described by van der Hoeven ('41), Poey ('56, '58), Gegenbaur ('01), and Wieder-

[3] These tests were made by Winkler method for determining the amount of dissolved oxygen in water. This method, although perhaps not entirely beyond experimental errors, is very satisfactory for the use to which it was put in this work. It becomes quite accurate when a considerable number of like individuals are tested and an average taken to represent the oxygen consumption of such specimens.

sheim ('07). There is a double row of trabeculae or muscular strands (figs. 2 and 3) in the swim bladder of an adult, one row of about thirty on each side. Each trabecula extends

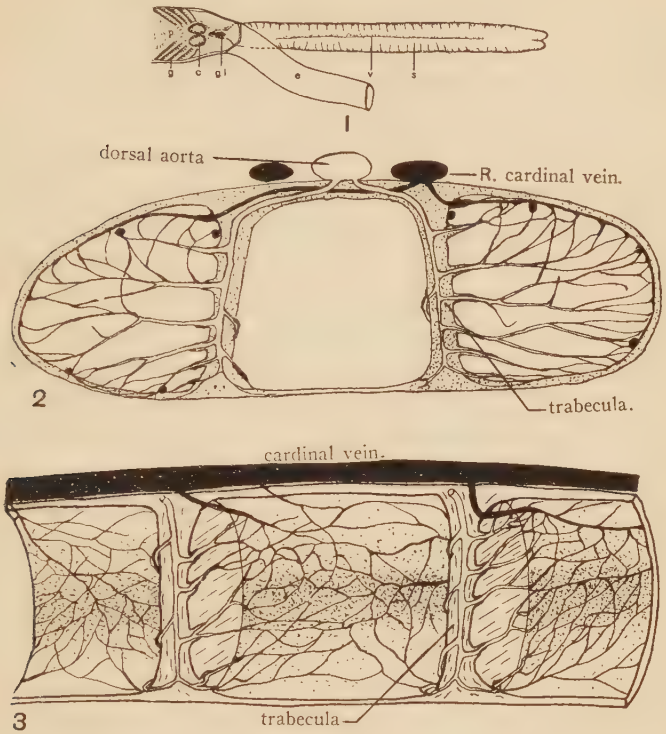

Fig. 1 Swim bladder, ventral view, showing esophagus and pharyngeal region with ventral wall removed. *c*, cartilaginous pad; *e*, esophagus; *g*, gills; *gl*, glottis; *p*, pharynx; *s*, swim bladder; *v*, ventral surface.

Fig. 2 Swim bladder of Lepidosteus in cross-section to show trabeculae, septa, and blood vessels.

Fig. 3 Swim bladder of Lepidosteus in longitudinal section through the level of the right postcardinal vein to show trabeculae, septa, and blood supply.

from the dorsal to the ventral wall, somewhat lateral to the median line of the bladder, leaving a space in the middle between the two rows. A thin septum stretches laterally from each trabecula and attaches to the lateral wall of the swim bladder. The trabeculae carry blood vessels which

branch out to the septa and walls. The septa divide both lateral portions of the organ into numerous chambers. The inner surface of the wall itself shows many small pockets or 'alveoli,' and was originally described as cellular and spongy in appearance. The writer ('25) has found the muscular tissue of the walls in well-developed embryos to consist primarily of striated fibers(24). Hence, one might suppose the organ to be under voluntary control.

The question of the vascularization of the swim bladder has been one of interest for many years and has a direct bearing upon the problem at hand. In order to use the swim bladder as a means of respiration, the blood supply of the organ must be such that a large supply can pass to its walls, spread out in the capillaries, which are exposed to the air on the interior, then be collected by veins and carried to the heart. Such investigators as Müller ('42), Hyrtl ('52), Quekett ('44), and Poey ('56) have contributed to the existing knowledge concerning this question. The arterial blood supply comes to the swim bladder by way of the dorsal aorta, which is immediately dorsal to the bladder and in contact with its wall. There are numerous branches from the ventral side of the aorta leading through the wall of the swim bladder and into the trabeculae, from which they branch out to the septa and continue on to the lateral and dorsal walls, where they usually divide into capillaries. These are entirely exposed to the air within the organ. The branches of the aorta are usually paired, the members of each pair going to the trabeculae on their respective sides. The blood is collected from the capillaries in the septa and walls of the bladder by branches of the right postcardinal vein, carried into the vein, and thence to the heart. There are two postcardinal veins, one on the right side of the aorta and one on the left. The veins collecting the blood from the left side of the bladder pass over it dorsally, but ventral to the left postcardinal and aorta, then join the ventral side of the right postcardinal along with the veins from the right (figs. 2, 3). This arrangement has probably some embryological significance.

The anatomy of this organ, with its open ductus pneumaticus, rich blood supply, sac-like shape, and 'cellular' interior, would seem to point to a possible respiratory function.

SUPPLEMENTARY RESPIRATION

The method used for showing the existence of a supplementary respiration was to place the normal animals in limited amounts of water and allow them to remain in the same water for long periods of time. The oxygen content is very quickly lowered, the normal oxygen content at the start for most of the tests was about 5 or 6 cc. per liter, at the end of the first twenty-four hours it tested, on the average, 1.9 cc. per liter. The fish continued to live in this foul water with no apparent discomfort. At the end of twenty days, on the average, the fish would die, but only after the water had become extremely foul and putrid. The water was tested several times for oxygen during this period, and the amount present always corresponded very closely to the amount found at the end of the first day. This test has been repeated a number of times and the results coincide. The experiments where the screen was fastened below the surface of the water, so the animals could not come to the surface to 'break,' showed that the fish could live only four to six hours under these conditions. A number of animals were confined under the screen until they were overcome and turned ventral side up, then they were placed in fresh tank water where they revived and became perfectly normal. It was noticed that during such experiments the rate of opercular movement was increased by about one-third.

This would seem to show, beyond a doubt, that there is supplementary respiration, but it gives no proof as to which organ is capable of this function.

SWIM BLADDER AS THE ORGAN OF SUPPLEMENTARY RESPIRATION

To show that the swim bladder is the organ which is responsible for respiration when the oxygen content of the water is far too low for respiratory purposes, the swim blad-

der was injected with melted paraffin so as to make it functionless. The gills were already functionless on account of the low oxygen content of the water. Now, if the animals with the bladder plugged are able to live only a short time, it would seem conclusive that the swim bladder is the organ responsible for the supplementary respiration. This is what happened. The injected animals died in four to eight hours when placed in deoxygenated water, while control animals were able to live for days in the same water. Injected controls were able to live in normal tank water for at least ten days; consequently, the death of the experimental animals was not due merely to the mechanical effects of the plugging of the bladder. The opercular movements of the animals used in these experiments were likewise about one-third faster than the normal. The autopsies of the animals in all cases showed that the plug served very well in closing the entrance of the bladder, so that no air could be drawn in. The results of this experiment form a very close parallel to the preceding ones in which the animals were confined below the surface of the deoxygenated water. This would indicate that the swim bladder is the organ used for the supplementary respiration.

ANALYSES OF GAS FROM THE SWIM BLADDER

In order to get a more definite check upon the respiratory function of the organ, it seemed desirable to make analyses of the gas from the bladder. The methods for procuring the gas have been previously discussed. A large number of samples of the gas was secured from normal fish which were living in normal tank water (dissolved oxygen in the water averaged about 5 cc. per liter and the temperature of the water averaged 20°C.). Normally, the animals 'break' on the average of once every seven to eight minutes. The samples were taken at two different points in the respiration cycle of the animal; immediately following an inhalation and again well into the interval between inhalations. Sufficient data are given in table 1 to show the results.

These data show that, under normal conditions, the animals consume some oxygen from the swim bladder and liberate a certain amount of carbon dioxide into it. This differs from Mark's conclusions in that there is carbon-dioxide discharge into the organ, even under normal conditions. It is true that the amount of carbon dioxide liberated does not compen-

TABLE 1

Analyses of gases from the swim bladders of normal animals in normal tank water

IMMEDIATELY FOLLOWING INHALATION			IN INTERVAL BETWEEN INHALATIONS	
Oxygen	Carbon dioxide		Oxygen	Carbon dioxide
Per cent	*Per cent*		*Per cent*	*Per cent*
6.27	1.51		3.10	2.06
7.04	1.34		2.85	2.41
6.09	1.13		3.96	2.80
8.16	1.74		4.95	2.37
8.14	1.84		3.51	2.03
8.06	1.86		3.69	2.99
5.98	1.61		3.11	2.48
7.91	1.76		5.11	2.39
7.21	1.59	Averages	3.79	2.44

TABLE 2

Analyses of the gases from the swim bladders of animals immediately following inhalation. The fish had been disturbed and were very active

Oxygen		*Carbon dioxide*
Per cent		*Per cent*
8.68		2.28
7.10		2.58
8.54		2.30
7.98		2.41
8.02		2.33
8.06	Average	2.38

sate for the entire amount of oxygen taken up, so that his idea of the gills' eliminating the carbon dioxide under such conditions is correct to a certain degree. It is quite likely that his methods were not delicate enough to detect small amounts of the gas.

The data from disturbed and active fish given in table 2 show a difference from that of the normal quiet animals. Ap-

parently, the activity causes the carbon dioxide to accumulate in the swim bladder as shown in table 2. The average of 2.38 per cent of carbon dioxide is high, when compared with 1.59 per cent average for the normal quiet animals.

The results of analyses as shown in table 3 indicate that there is a steady exchange of gas under such conditions. It

TABLE 3

Analyses of gases from animals in normal tank water and analyses of gases from the same animals after they have been out in the air for three minutes without inhalation. The animals struggled considerably

IMMEDIATELY FOLLOWING INHALATION			THREE MINUTES LATER	
Oxygen	Carbon dioxide		Oxygen	Carbon dioxide
Per cent	*Per cent*		*Per cent*	*Per cent*
8.76	1.86		7.06	3.45
9.01	1.56		7.92	3.19
10.62	1.08		8.51	3.37
9.46	1.50	Averages	7.83	3.33

TABLE 4

Analyses of the gases from the swim bladders of animals exposed to the air for thirty minutes (very active)

IMMEDIATELY FOLLOWING INHALATION			IN INTERVAL BETWEEN INHALATIONS	
Oxygen	Carbon dioxide		Oxygen	Carbon dioxide
Per cent	*Per cent*		*Per cent*	*Per cent*
10.00	4.11		4.99	4.51
9.75	4.10		4.60	4.28
10.06	4.14		5.16	5.25
9.09	4.02		4.90	4.72
9.72	4.09	Averages	4.91	4.69

varies somewhat with different individuals and the amount of carbon dioxide does not quite approximate the amount of oxygen consumed, but it shows a rather constant rate of exchange.

Lepidosteus can endure long exposures to the air out of the water. A number of them were taken out of the water and kept out for thirty minutes, then samples of gas were taken from the swim bladder, as indicated in table 4. It is rather

difficult to get these samples, because the rate of aerial respiration is greatly increased, hence shortening the time between 'breaks.' The work of getting the samples must, therefore, be somewhat hurried. The rate of consumption of oxygen and discharge of carbon dioxide is not quite balanced, but, as explained, the time between 'breaks' does not allow for the most careful work in obtaining the samples, so that it may be that some of them were not quite perfect. Nevertheless, they show that the animal is able to carry on aerial respiration entirely apart from the branchial type.

TABLE 5

Analyses of gases from the swim bladders of animals kept in foul water

IMMEDIATELY FOLLOWING INHALATION		IN INTERVAL BETWEEN INHALATIONS	
Oxygen	Carbon dioxide	Oxygen	Carbon dioxide
Per cent	*Per cent*	*Per cent*	*Per cent*
8.40	2.98	4.70	5.01
8.60	3.01	5.02	5.23
9.04	3.09	4.91	5.40
8.68	3.02	4.87	5.03
8.99	2.99	4.93	4.90
8.74	3.02 Averages	4.89	5.11

A series of the fish was run in deoxygenated water and tests made upon the samples taken as before, immediately following inhalations and in the intervals between them. Here again it was necessary to work rapidly in getting the samples, on account of the increased rate of aerial respiration. The results are quite similar to those in fish taken entirely out of the water.

These data are enough to indicate clearly that both oxygen is consumed and carbon dioxide discharged by the fish in its use of the swim bladder as a respiratory organ. It is true that the carbon dioxide does not begin to appear in large quantities until after the water in which the animal is living becomes more or less saturated, but it does accumulate in the swim bladder in considerable amounts as the experiment goes

on. It seems that even then the oxygen-carbon dioxide balance is not reached, so it is probable that a part of the carbon dioxide is still being discharged through the gills or being absorbed in the water. Apparently, that is what happens

TABLE 6

Analyses of gas from above the water in an air-tight chamber where a fish is living

TIME	OXYGEN	CARBON DIOXIDE
Animal 1 (large)		
	Per cent	*Per cent*
1:00	Normal air	Normal air
4:00	12.16	2.95
6:00	10.50	3.37
7:30	7.48	4.18
The fish was taken out in good condition.		
Animal 2 (medium)		
3:00	Normal air	Normal air
5:00	16.16	0.43
6:10	14.57	1.55
7:10	13.23	2.64
8:10	12.39	2.90
10:20	10.68	3.66
Animal 3 (large)		
8:00	Normal air	Normal air
10:00	14.60	0.87
11:00	11.98	2.52
12:00	10.82	3.11
2:00	7.92	4.54
All of the fish finished the experiment in good condition.		

during the first two hours, since the tests show but small amounts of carbon dioxide for this period, but at the third hour it takes a sudden jump. The increase seems to come when the water becomes nearly saturated. This probably explains Mark's conclusion, that "the carbon dioxide is discharged entirely through the gills." For fear of killing his

animals, he did not carry his experiments far enough to get the more essential latter part where the real gas exchange is shown. Furthermore, Mark's method for determining the components of the gas quantitatively was not delicate enough to show the small amount of carbon dioxide present.

CAPACITY OF THE SWIM BLADDER

At the rate which the animal respires, the capacity of the swim bladder was found to be great enough to allow a sufficient gas exchange for the needs of the body. Its capacity in the animals used was from 50 to 100 cc., depending upon the size of the animal. The gram-hour rate of oxygen consumption of Lepidosteus has been found to be, on the average, 0.0369 cc. The normal number of 'breaks' averages about eight per hour and 600 grams is the average weight of an animal whose swim bladder has a capacity of 50 cc. Air contains about 20.9 per cent oxygen. The gas exchange in the swim bladder for each 'break' averages about 50 per cent. The computation would be as follows:

600 grams × 0.0369 cc. equals 22.14 cc., or total oxygen consumed per fish per hour.

8 × 50 equals 400 cc., or total amount of air available per hour.

400 cc. × 20 per cent equals 80 cc., or oxygen that would be available if the exchange was perfect at every 'break.'

80 cc. × 50 per cent equals 40 cc., or available oxygen per hour.

When the 40 cc. of available oxygen is compared with the twenty-two and a fraction consumed per hour, it is seen that the possible amount of oxygen furnished by the swim bladder is sufficient to supply the needs of the fish.

DISCUSSION

In all of the previous work upon this subject as reported in the literature, the postulations, that the swim bladder has respiratory function, have been based upon anatomical studies and more or less superficial observations, e.g., the cellular and vascular structure of the bladder, its connection with the pharynx, and that the animal seems to draw air into the mouth at intervals. Müller ('42) and Hyrtl ('52), both study-

ing the anatomy and vascularization of the swim bladder, did not consider it a respiratory organ, because they failed to realize that, if the water in which the animal was living did not contain oxygen, the blood coming from the gills would carry no more oxygen than blood in any other part of the body.

There has been a theory constructed for the origin of air-breathing vertebrates and their transition from aquatic to terrestrial animals. One basic factor in this story, which seems to have been formulated by Wilder ('77) and Morris ('85, '92) is the development of an organ in the fish capable of using atmospheric air in oxygenating the blood. From the rather indirect anatomical evidence, plus the fact that the animals seem to inhale and expel air through the mouth, certain of the ganoids and lung-fishes have been supposed to put the swim bladder to this use.

Mark ('90) made a start in the study of this subject with three experiments, but he did not carry the work far enough to make it conclusive. The conclusions which he did draw apply only to fish in perfectly normal conditions and then only partially, because it has been shown in this paper that he was mistaken about the carbon dioxide being entirely absent in the exhaled gas. Many authors have used a number of these facts to build theories for the phylogenetic development of the lungs. The writer feels that, by adding the data which are given in this paper, the hypotheses are placed upon a much firmer foundation and give additional evidence supporting the belief that the ganoids are the key to the origin of air-breathing vertebrates.

The anatomy of the swim bladder of Lepidosteus has been carefully studied, and, along with this, a thorough review has been made of the literature upon the subject. It is interesting to find that a number of investigators, from anatomical data alone, have declared that this organ is one of respiration in several genera of fish, including Lepidosteus. The present studies of the blood supply of the swim bladder of Lepidosteus show that it is adequate and satisfactorily arranged to

enable the organ to carry on respiration. It has long been known that the animal has an open ductus pneumaticus leading from the pharynx to the swim bladder. This connection would make it possible for the fish to force air from one of these cavities to the other, i.e., when air is gulped into the mouth it can be carried to the swim bladder and later expelled. So far as is known, up until now, it has never been proved that air is actually conveyed to the swim bladder in this way.

In this paper it is shown experimentally that Lepidosteus actually inhales atmospheric air into the swim bladder, where it serves in supplementing the branchial respiration, both as the usual thing and to a much greater extent when the oxygen content of the water is low. The methods used in showing this have been: to carry on experiments with normal animals in deoxygenated water, allowing part of them to come to the surface for air, and confining others below the surface; to inject melted paraffin through the glottis into the swim bladder of live animals and run them against controls in normal and deoxygenated water; to analyze gas from the swim bladder of the live fish under various conditions, immediately following inhalation of air and again in the interval between inhalations; and, analysis of air from above deoxygenated water in an air-tight chamber where an animal is living.

The fact that normal animals live twenty days in a small amount of deoxygenated water if allowed to come to the surface and gulp in air, but are killed in five or six hours if confined below the surface, while animals live for days with no apparent discomfort when confined by a screen, if a current of oxygenated water passes through the container, shows definitely that there is supplementary respiration. The really significant point is that animals whose swim bladder has been injected with paraffin die in four to eight hours when placed in deoxygenated water, while control animals live for days in the same water, and injected controls are able to live in normal tank water for at least ten days. This fact is proof that the swim bladder is the organ which is used for supplementary respiration in this fish. The gram-hour rate of con-

sumption of oxygen by the fish has been worked out and shows that the volume of the swim bladder and the rate of inhalations are great enough to supply sufficient air for the needs of the body.

The analyses of gases from the swim bladder also confirm the above conclusion, that the swim bladder is the organ of supplementary respiration. Analyses of the gas from normal animals in normal tank water show a noticeable decrease in oxygen and an increase in carbon dioxide between inhalations, but when these tests are performed upon animals which have either been kept in foul water or exposed to the air for a period of time there was a marked decrease in oxygen and increase in carbon dioxide. The analyses of the air from above the oil-covered water where the animal is confined in the air-tight vessel also gave results which show that the fish uses this air in respiration.

The results of this investigation have shown beyond a doubt that Lepidosteus really makes use of the swim bladder as an organ of respiration. Therefore, it is felt that the results and explanations offered in this paper give additional information concerning the swim bladder-lung situation, which is a problem of fundamental biological interest.

The writer is greatly indebted to Dr. F. A. Stromsten for his careful supervision, and to Dr. G. L. Houser and Dr. W. W. Swingle for their helpful suggestions and kindly interest in the work. The U. S. Biological Laboratory at Fairport, Iowa, very kindly and obligingly supplied a large number of the animals.

SUMMARY AND CONCLUSIONS

1. The anatomy of the swim bladder of Lepidosteus indicates its possible use as a respiratory organ.

2. Lepidosteus inhales atmospheric air by coming to the surface of the water and gulping it into the mouth.

3. No experimental proof has heretofore been presented to show that this inhaled air is carried into the swim bladder.

4. There are theories which hold that the swim bladder is a respiratory organ in Lepidosteus. These are based upon its general anatomy, vascularization, and the fact that the fish gulps air into the mouth.

5. Normal animals may live for twenty days in foul water if allowed to reach the surface and 'break,' but they die in five or six hours if confined below the surface. Control animals live for days when thus confined if a current of oxygenated water passes through the container. This indicates that the fish has means of supplementary respiration.

6. The gram-hour rate of oxygen consumption by the fish shows that the capacity of the swim bladder and the rate of inhalations are great enough to supply sufficient air for the needs of the body.

7. Animals whose swim bladders have been injected with paraffin can live only four to eight hours in foul water, but are able to live at least ten days in normal tank water. These results clearly indicate that this fish uses the swim bladder as the organ of supplementary respiration.

8. Further confirmatory evidence of aerial respiration is shown by the analysis of gas from the swim bladder of the fish.

9. Analysis of air from above deoxygenated water where an animal is living likewise gave results which confirm the above statement.

10. Lepidosteus utilizes the swim bladder as an organ of respiration under normal conditions, and to a greater extent when the oxygen content of the water is low.

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SULPHATE RETENTION IN DOGS FOLLOWING BILATERAL ADRENAL EXTIRPATION[1]

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THE results of previous work by the senior writer (1926 and 1927) and his co-workers (Zwemer, 1927; Corey, 1927 Yonkman, 1927; Swingle and Eisenman, 1927) on the functional significance of the suprarenal cortex indicated that probably the cause of death in double-operated cats and dogs is acid intoxication due to retention of acids formed in the course of normal metabolism. The acidosis is apparently not due to the formation of abnormal acids within the organism, but primarily to defective elimination of non-volatile acids. In the earlier work, attention was called to the resemblance between the acid intoxication resulting from adrenal insufficiency and that occurring in the terminal stages of chronic interstitial nephritis, eq. vremia.

In a study of the acid-base equilibrium of adrenalectomized cats, Swingle and Eisenman (1927) determined the serum protein, serum carbon dioxide, serum chloride, inorganic phosphorus, total base, and pH. The acid values of protein, bicarbonate, chloride, and phosphorus were calculated. Total acid was taken to be the sum of all the determined acids, while organic acid was calculated by subtracting total acid from total base and included the sulphate ion. Sulphur determinations were not made because of the large quantity of blood required in addition to that necessary for the other tests. It was not deemed expedient to subject the experimental animals to loss of such considerable amounts of blood.

The results of the acid-base equilibrium tests on normal and operated cats showed quite clearly that the animals showing marked symptoms of adrenal insufficiency were suffering from an uncompensated non-volatile acidosis. According to the data available, the

[1] Part of the expenses of this investigation were defrayed by a grant from the Bache fund of the National Academy of Science.

acidosis was attributed to an increase in phosphoric and undetermined organic acids. However, it was evident that the marked fall in bicarbonate observed in our double-operated animals was not occasioned to any considerable extent by the rise in inorganic phosphorus, which, although marked, never exceeded 2 millimols, whereas the carbon dioxide fell from 6 to 10 millimols. We stated that "increase in organic acid [which in our data included the sulphate ion] is the one change of sufficient magnitude to be responsible for the bicarbonate fall."

Recent experiments performed in this laboratory on dogs by Yonkman (1927) have likewise shown that the rise in inorganic phosphorus is not sufficient to account for the degree of acidosis observed.

Further efforts on our part to determine the nature of the organic acid or acids responsible for the fall in bicarbonate ended in failure; hence, it seemed worth while to test for sulphate retention to see if the sulphate ion could be responsible for the observed changes, since in the original work the sulphate ion was lumped together with organic acid. The idea seemed especially likely to prove fruitful since earlier experiments by Swingle (1927) had shown that the kidney function is abnormal in adrenal insufficiency. The work of Denis (1923) and others had also demonstrated that the sulphate ion is apparently difficult for the kidney to eliminate even under normal conditions and is retained in considerable quantities in certain types of nephritis.

Dogs were chosen for experimental animals owing to the ease with which they can be bled and the quantities of blood that can be taken without affecting the animal in any way.

The blood was drawn directly from the heart in all cases. In the earlier experiments, the blood inorganic sulphate of the normal unoperated dogs was determined. It soon became evident, however, that unilaterally operated dogs are perfectly normal and can be used for controls. Later on in the work, the control blood was taken from bilaterally operated animals which did not present symptoms. This is a legitimate procedure since double-operated dogs remain normal for several days following removal of the second adrenal, and during this period abnormal blood changes do not occur. Once the animals

refuse food and seem depressed and moody, the normal period is over. Only dogs surviving the second operation from 4 to 6 days were employed in the present work. Fifteen dogs survived less than this period and were discarded for this experiment.

The method used for determining blood sulphate is that of Denis and Reed (1926) and is as follows: 25 cc. of blood was drawn directly from the heart into a 50 cc. Leur syringe and immediately placed in a small flask containing citrate. To this was added 55 cc. of redistilled H_2O, and 20 cc. of a 20 per cent trichloracetic acid solution. This was shaken well and allowed to stand for 1 hour and

TABLE I

THE INORGANIC SULPHATE OF NORMAL DOG BLOOD

NORMAL UNOPERATED		UNILATERALLY OPERATED	
Animal No.	Sulphur per 100 Cc. (Mgm.)	Animal No.	Sulphur per 100 Cc. (Mgm.)
1	0.8	12	2.4
2	1.5	13	1.6
3	0.9	14	2.8
4	1.0	15	2.5
5	1.1	16	3.1
6	1.8	17	2.3
7	1.1	18	2.1
8	2.3	19	2.2
9	0.9	20	3.2
10	1.0	21	2.1

filtered. Twenty-five cc. of clear filtrate were taken and to this were added 75 cc. of redistilled H_2O and 20 cc. of 5 per cent barium chloride. This was permitted to stand 12 hours. It was then transferred to a tarred Gooch crucible with asbestos mat, washed, dried, and ignited to constant weight. Blanks were run with all blood determinations. Merck's trichloracetic acid was used containing 0.0495 per cent sulphur. This was taken into consideration when making the calculations.

The diet of the dogs was the same before and after operation. It consisted of finely ground raw beef, bones, table scraps, and an occasional half-pint of milk. The animals were mongrels averaging 6–9 kilos in weight.

Table I shows the amount of inorganic sulphur (as sulphate) per

100 cc. of blood in normal and unilaterally operated dogs 5–7 days after removal of the right adrenal.

The unilaterally operated animals were to all appearances normal, and in no way could they be distinguished from the unoperated ones. The slight increase in inorganic sulphate of these dogs is probably of no great significance since it is within the range of error of the method employed. However, it is of interest in this connection that two other investigators recently reported a slight increase in blood sulphur of unilaterally operated dogs (Loeper *et al.*, 1926). These investigators appear not to have made sulphur determinations

TABLE II

THE INORGANIC SULPHATE OF THE BLOOD OF ADRENALECTOMIZED DOGS

ANIMAL NO.	UNILATERALLY OPERATED	BILATERALLY OPERATED	REMARKS
	Sulphur per 100 Cc. (Mgm.)	Sulphur per 100 Cc. (Mgm.)	
10	2.1	11.2	Very weak; marked acidosis
11	2.0	10.4	Weak; acidosis
12	1.7	12.7	Weak; acidosis present
13	1.4	10.2	Staggers about; acidosis
16	0.9	8.0	Animal weak; acidosis
19	1.0	11.9	Acidosis; verging on coma
22	1.1	7.2	Weak; could walk about; acidosis
23	1.8	9.1	Weak; acidosis
24	2.1	13.0	Verging on coma; acidosis

on bilaterally operated animals presenting symptoms of adrenal insufficiency.

When serious symptoms of adrenal insufficiency intervene following bilateral gland removal, the rise in inorganic sulphate becomes apparent. During the "fore" period after extirpation of the remaining gland, the interval during which the dog seems normal, no increase in sulphate is noticeable. The rise is most abrupt when the animal is verging on coma. Inorganic phosphate and inorganic sulphate behave similarly with respect to the stage of adrenal insufficiency during which the greatest increases take place. Increased concentration of phosphate and sulphate in the blood parallels the increase in severity of the adrenal insufficiency symptoms and the degree of acidosis. Table II shows the increase in blood sulphate following the onset of serious adrenal symptoms.

The rise in inorganic sulphate in the blood of double-operated dogs presenting marked symptoms is quite evident from examination of Table II. The animals were bled when they showed symptoms of acid intoxication but while they were still conscious and were able to move about. The amount of blood necessary for our determinations was considerable; and usually additional amounts were taken for pH, sugar, CO_2, and phosphorus—necessitating about 50 cc. altogether. We noted that adrenalectomized dogs presenting marked symptoms of acid intoxication rarely survived but a few hours following removal of 50 cc. of blood. The symptoms were always aggravated by bleeding. There can be no question but that bleeding shortens the life-span of adrenalectomized dogs and cats.

In several animals we were able to make determinations of the inorganic phosphorus, CO_2, pH, and sugar simultaneously with inorganic sulphate. The results are shown in Table III.

DISCUSSION

The results of this investigation show that sulphate retention occurs following bilateral adrenal removal in dogs. The rise in sulphate is especially pronounced following the onset of serious symptoms of acid intoxication, and parallels the rise in inorganic phosphate and nitrogen.

Denis (1921) reports that the inorganic sulphate of the blood of various animals varies between 1.8 mgm. and 4 mgm. of sulphur per 100 cc., whereas in normal human blood the quantity of inorganic sulphate is much less, varying from 1.0 to 0.5 mgm. S. per 100 cc. In nephritis with nitrogen retention, figures for inorganic sulphate as high as 12–16 mgm. S. per 100 cc. are not uncommon. In those types of nephritis characterized by slight nitrogen retention, the retention of sulphate is much less. It has also been demonstrated by Denis that the experimentally induced nephritis in rabbits which follows injections of large doses of uranium nitrate is characterized by marked sulphate retention. The sulphate ion is apparently excreted more slowly by the kidneys than are the other ions which constitute the normal blood salts. Consequently any malfunctioning of the acid-elimination function of the kidney mechanism leads to sulphate retention. In so far as the writers are aware, nephritis and adrenal

TABLE III

BLOOD CHANGES FOLLOWING ADRENALECTOMY IN DOGS

Animal No.	Unilaterally Operated	Sulphate per 100 Cc. (Mgm.S.)	Bilaterally Operated	First Symptoms Noticed	Survival Period (Days)	Sulphate per 100 Cc. (Mgm.S.)	pH	CO_2 Capacity (Vols. per Cent)	CO_2 Content (Vols. per Cent)	P (Mgm.)	Sugar per 100 Cc. (Mgm.)	Condition of Animal When Bled
26.....	6– 8 at 10 A.M.	0.9	6–15 at 4 P.M.	6–18 at 1 P.M.	5	11.9	7.26	27	20.6	10	56	Weak
27.....	6– 9 at 11 A.M.	1.2	6–16 at 2 P.M.	6–19 at 9 P.M.	6	10.7	7.25	25	19.2	9.2	50	Weak
29.....	5–18 at 2 P.M.	1.4	5–26 at 9 A.M.	5–29 at 10 A.M.	$5\frac{1}{2}$	11.1	7.22	22	19.0	12.1	45	Verging on coma
31.....	5–21 at 3 P.M.	1.2	5–29 at 3 P.M.	6– 2 at 11 A.M.	5	9.4	7.27	27	21.5	13.1	57	Weak
33.....	5–24 at 11 A.M.	1.2	5–30 at 9 A.M.	6– 3 at 8 A.M.	$6\frac{1}{2}$	12.2	7.25	26	21.4	10.2	62	Weak
34*....	5–20 at 10 A.M.	1.4	5–28 at 5 P.M.	5–31 at 2 P.M.	$4\frac{1}{2}$	9.1	7.27	32.1	28.1	12.5	53	Weak

* Venous blood used.

insufficiency are the only pathological conditions reported which are characterized by retention of inorganic sulphate in the blood.

It was stated earlier in this paper that previous studies on the acid-base equilibrium of bilaterally adrenalectomized cats led Swingle and Eisenman (1927) to the belief that the acid intoxication observed was due to rise in inorganic phosphate and undetermined organic acids. The sulphate ion was included with organic acid owing to the difficulty of testing for it separately. The results obtained in the present work lead us to believe that retention of the sulphate ion plays a considerable rôle in the acid intoxication of adrenal insufficiency. Whether or not the rise in inorganic phosphorus and sulphate are sufficient to account entirely for the degree of acidosis observed in adrenal insufficiency, the writers are not prepared to say. It seems likely that the fall in bicarbonate is to a considerable extent due to retention of these two acids. There is, in all likelihood, also some increase in organic acid or acids of undetermined nature.

Loeper and his collaborators (1926) report an increase of blood sulphur in three cases of Addison's disease; the proportion of the oxidized sulphur was reduced, while that of the neutral sulphur was increased. They state, furthermore, that the blood of the suprarenal artery contains much more sulphur than that of the corresponding vein and that in the artery the proportion of oxidized to neutral sulphur is lower than in the vein. They conclude that the suprarenal glands retain a part of the blood sulphur, especially the neutral.

The question of the relation of the suprarenal glands to sulphur metabolism is a promising field for investigation, but at the present time practically nothing is known concerning it. Our own experiments on sulphate retention following adrenal extirpation point to the conclusion that kidney impairment soon follows removal of the cortex leading to sulphate and phosphate retention, and they throw no light upon the rôle of these glands in blood sulphur regulation.

SUMMARY AND CONCLUSION

1. Retention of inorganic sulphate occurs following the onset of serious symptoms of adrenal insufficiency in dogs.

2. Unilaterally operated dogs and bilaterally operated animals

showing no symptoms have normal amounts of inorganic sulphate in the blood.

3. The increase in inorganic sulphate parallels the increase in inorganic phosphate and nitrogen.

4. It is probable that the retention of inorganic phosphate and inorganic sulphate is sufficient to account in large part at least for the degree of acid intoxication observed in adrenal insufficiency in dogs.

5. Organic acid is probably not so important a factor in inducing the fall in bicarbonate as the phosphate and sulphate.

6. With the exception of those types of nephritis characterized by nitrogen retention and acidosis, adrenal insufficiency is, so far as we are aware, the only other condition where sulphate retention occurs.

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AN EXPERIMENTAL STUDY OF OVARIAN REGENERATION IN MICE[1]

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THE entire question of regeneration long has held a place of prime importance as a problem for investigation. As displayed in lower forms, regeneration has been the subject of considerable experimentation; and it has been quite clearly demonstrated that many types possess a remarkable capacity for growth of new tissue or—more strictly—a redifferentiation of old tissue to subserve a new function. It is a matter of common knowledge that this is shown to a striking degree in the segmented worms, in the echinoids, and—among vertebrates—in the amphibians.

To what degree the conclusions derived from observations on lower forms may apply to mammals seems still a matter for speculation. In a general way, it has not been satisfactorily demonstrated that tissues of the higher Amniota, once differentiated, can adapt themselves to serve a different function—the plasticity displayed by lower forms evidently having been lost somewhere along the line of phylogenetic development. However, Davenport (1925), working with mice, reported a remarkable degree of regenerative capacity of ovarian tissue following ovariectomy in these rodents, regeneration occurring in 64 per cent of his animals following removal of the gonads, in many cases functional ovaries being developed. The experimental period ranged in time from 8 to 45 weeks, and he based his conclusions upon the fact that post-mortem examination revealed distinct ovarian masses on the site of the original ovaries, and in many cases embryos were found *in utero*.

Should Davenport's conclusions be correct, they would have considerable significance in their bearing upon modern ideas concerning germ-cell origin, and the question is revived: Does germ-

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cell tissue in vertebrates arise *de novo* from the peritoneum, or is it set aside at an early stage in embryonic development, not to be duplicated or replaced later by transformation of differentiated peritoneal cells? The former idea, that of a "germinal epithelium," was advanced by Waldeyer as far back as 1870; and the latter view, one of an "early segregation," was first suggested by Nussbaum in 1880. While both of these theories have received staunch support (the former—to cite but a few—from Hoffmann, 1886; Dustin, 1907; Kuschakewitsch, 1910; von Berenberg-Gossler, 1912; Gatenby, 1916; and Essenberg, 1926; and the latter from Beard, 1900; Allen, 1906; Dodds, 1910; Swift, 1914–16; Jordan, 1917; Firket, 1920; Okkelberg, 1921, and Swingle, 1921, 1926), the question still is unsettled, with the weight of evidence in favor of the idea of an early segregation of germ-cell substance. If Davenport's report is correct, however, and regeneration of ovarian tissue from non-gonadic substance does occur in mice, such evidence would lend unequivocal support to the theory of a peritoneal origin. In his data, however, no histological evidence was advanced to show that all of the original ovary was removed in each case, and it seems essential that such evidence be established before it can be stated definitely whether regenerated ovarian tissue actually arises from the coelomic epithelium in mice, or from fragments of the original germ gland inadvertently left *in situ*.

Recently Domm (1927), in working on the problem of sex-inversion in the domestic fowl, was led to perform a large series of gonadectomies in hens. Subsequent examinations revealed in a number of instances a regeneration of ovarian substance, and these he attributed to incompleteness of removal of the original ovary, in consequence of the difficulties inherent in such an operation in the fowl. Some operations were known to be incomplete at time of removal, and regeneration was to be expected. In a few instances, however, regeneration followed apparently complete ovariotomy, despite the fact that the ovarian region had been thoroughly cauterized, the tissue regenerated being functional to such an extent as seriously to interfere with the object of operation. With regard to these latter cases Domm states (page 98) that "In such cases the mass of ovarian tissue which remained behind must have been ex-

ceedingly small—in fact, in some cases one would wager that they were so small as to be almost invisible. Nevertheless, such fragments grow and hypertrophy and counteract the effects of complete ovariotomy." In some instances onset of hemorrhage necessitated the deliberate leaving *in situ* of a small fragment of ovary. In each case this was found to undergo hypertrophy; and, although the mass left was very small, in a few months it had attained the size of a normal ovary.

The experimental work upon which the following report is based was begun with the object in view of ascertaining whether or not there is any regeneration of ovarian tissue in mice following complete ovariectomy, basing any conclusions drawn upon histological study. Such evidence would show definitely two things: (1) whether or not all of the original ovary had been removed—a factor of prime importance—and (2) whether or not the regenerated tissue actually is ovarian in nature.

The writer wishes to acknowledge his indebtedness to Dr. W. W. Swingle for his suggestion of this problem and for his advice and criticism throughout the course of the work.

MATERIAL AND METHODS

Adult female mice of healthy stock were selected for operation. While no stress was laid upon age or strain, the animals for the most part were young adults, the greater portion of them never having had litters. The operative procedure was varied somewhat, care being exercised, however, that as little non-ovarian tissue as possible be removed, in order that regeneration, should any occur, might not be hindered. In the greater percentage of cases the capsule was split and the ovary cut off at the hilus; in some instances the capsule and a short section of the stalk were included as well in removal. All extirpated material was fixed in Bouin's fluid and preserved, thus being rendered available for subsequent microscopic examination, should the need arise.

Ninety-six animals were bilaterally operated, by means of small lumbolateral incisions. Both ovaries were removed at one time, with the use of finely pointed iridectomy scissors, under a binocular dissecting microscope. Pinching off of blood vessels usually sufficed

to prevent hemorrhage, and very little bleeding occurred. The wounds were sutured by means of a single stitch in the muscle layer and in the skin; although few aseptic precautions were observed, no cases of infection occurred, and healing took place very rapidly. Anesthesia mortality, to which mice seem particularly susceptible, was practically eliminated through frequent swabbing of the throat, for which purpose toothpicks wrapped in cotton were employed. Such procedure served to check a tendency to choke through excessive salivation. The operated mice were kept on a balanced ration

TABLE I

SUMMARY OF CASES EXAMINED

Interval between Operation and Examination (Weeks)	No. Examined	No. Regeneration*	Regeneration
7	2	2	0
8	13	13	0
9	39	35	4
10	16	16	0
11	1	1	0
15	2	2	0
16	8	6	2
21	17	16	1
22	12	11	1
23	21	21	0
24	4	4	0
25	6	6	0
26	11	10	1
28	2	2	0
32	2	2	0

* Of the list in the "no regeneration" column, several showed a growth of suspicious appearing masses on the site of the original ovary; in each instance sections showed these to be non-gonadic in nature, consisting chiefly of connective and of lymphoid tissue.

(cereals, greens, milk, etc.) and were allowed to run together with unoperated females and with males.

Many of the operated animals were reopened at intervals during succeeding weeks (see Table I), and the ovarian region was examined for evidences of regeneration. All suspicious masses were removed, sectioned, and examined for traces of ovarian tissue: in a number of instances small bladderlike cysts had developed on the site of the original ovary, but sections of these revealed no ovarian tissue. Complete bilateral castration was invariably followed by atrophy of the genitalia—a condition which remained persistent.

The tubes and uterus on subsequent examination were found to be small and anemic, and the external genital parts shrunken and dry in appearance. A uterus normal in size and appearance invariably had as its sequel the discovery of a fragment of ovarian tissue, usually embedded in fat and rather heavily encapsulated.

RESULTS AND DISCUSSION

The results of the examinations are arranged in tabular form in Table I and show that, out of 96 operated cases (duplicated in many instances in the table as a result of repeated examinations), there were 9 instances in which germ-cell or ovarian tissue was discovered, indicative of regeneration. As recorded in the protocols, the histories of these regenerated cases are as follows:

OPERATION, February 6

6A. Right ovary, capsule, and some mesentery removed.

6B. Left ovary and capsule removed; ovary somewhat mutilated. Considerable bleeding.

Examined July 12 (22 weeks)

6A. Uterus and tube normal in size and appearance; a large dark mass on site of original ovary, somewhat larger than average ovary, with several whitish patches. Removed and sectioned. Ovarian.

6B. No evidence of ovarian tissue.

(Examination of original ovaries showed 6A to be deeply cut, and split in the center; 6B was entire.)

OPERATION, February 7

7A. Right ovary removed, but doubtful if entire. Capsule not removed.

7B. Left ovary removed; badly mutilated. Capsule removed.

Examined August 9 (26 weeks)

7A. Tube normal in size and vascularity; no evidence of ovarian tissue.

7B. Small mass of ovarian tissue on original site.

(Examination of original ovaries: 7A, some mutilated tissue in hilus region; 7B, ovary cut nearly in two.)

OPERATION, February 9

18A. Right ovary entirely removed, plus capsule and some mesentery.

18B. Left ovary removed, without capsule; cut off at hilus. Bad hemorrhage.

Examined April 18 (9 weeks)

18A. Very evident mass of ovarian tissue on old site. Removed and sectioned. Ovarian.

18B. A somewhat smaller mass of tissue. Sectioned. Ovarian.

Autopsied July 14 (22 weeks)

18A and B. Nothing found on either side. Uteri and tubes atrophied.

Operation, February 11

19A. Right ovary cut off at hilus; capsule removed, with some mesentery.

19B. Left ovary removed. Left a small lobe on one side. Cut off at hilus.

Examined April 18 (9 weeks)

19A. No evident ovarian tissue; oviduct normal.

19B. Small round fragment on site of original ovary which appeared to consist of follicles. Not romoved.

Autopsied July 12 (22 weeks)

19A. Same as the foregoing.

19B. Two fairly large white bodies embedded in fingers of tube. Sectioned and found to be typical lutein cells.

(Examination of original ovaries: 19A, entire; 19B, minus a small lobe.)

Operation, February 16

34A. Right ovary removed entire, plus capsule.

34B. Left ovary removed entire, plus capsule. Latter slit through at one place.

Examined April 22 (9 weeks)

34A. No evident ovarian tissue. Uterus normal in size.

34B. Adhesions; two small capsules of very evident ovarian tissue on old site, and a larger mass adherent to tube.

(Examination of original ovaries revealed: 34A, entire; 34B, with a cut on one side and a small band of protruding tissue.)

Operation, February 19

44A. Right ovary removed entire, cut off at hilus. Little bleeding.

44B. Left ovary removed entire. Capsule split off.

Examined April 20 (8 weeks)

44A. Adhesions; fat. No evident ovarian tissue. Uterus fairly large.

44B. Same as 44A.

Autopsied July 14 (21 weeks)

44A. Adhesions. A small mass of evident ovarian tissue deeply embedded in fat. Removed and sectioned. Ovarian.

44B. No evident ovarian tissue. Uterus and tubes normal.

(Examination of original ovaries: both appear entire, with no cuts or abrasions.)

Operation, December 31, Series B

BA. Right ovary removed entire, plus capsule. Cut off at hilus.

BB. Left ovary removed entire, plus capsule and some mesentery. Cut off at hilus.

Autopsied April 22 (16 weeks)

BA. Adhesions. Very evident ovarian mass embedded in fat around tube. Not preserved.

BB. Adhesions. A very small mass of ovarian tissue embedded in fat near tube. Not preserved. Tubes normal.

(Examination of original ovaries: both appear entire.)

Following the discovery of apparent regeneration, the origina ovaries were subjected to careful scrutiny, and in some cases were sectioned, to make certain that removal had been complete. Examination of original tissue in four instances (Cases 6A, 7B, 19B, 34B) would indicate that complete extirpation was doubtful—in some cases the material being somewhat mutilated. Under such circumstances, one cannot be certain that all of the original ovary had been removed; and, in view of the remarkable regenerative capacity (see below) of small bits of germ-cell tissue, it would be hazardous to assume that such regeneration had occurred from non-gonadic tissue.

Five cases remain (18A, 18B, 44A, BA, BB) of which the notes show apparently complete removal of the original ovary, examination revealing no cuts or abrasions. Such a small percentage (5 out of 96) of apparent regenerations from non-gonadic tissue gives rise to a reasonable doubt as to the nature of their origin. It seems by no means unlikely, even under the most careful operative precautions, that microscopic bits of germ-cell tissue may have been left *in situ*, particularly in the hilus region.

PARTIAL OPERATIONS

As a check on the possibilities of the regenerative capacity of ovarian tissue, a series of five partial operations was performed, to determine to what extent regeneration occurs following incomplete removal. The case histories of these are given below:

OPERATION, March 20, Series C

47. Right ovary about three-fourths removed. (Ovary rather small.) None of stalk and no mesentery removed. Some bleeding. Left ovary not removed.

Examined April 23 (5 weeks)

Right ovary average in size, surrounded by a rather heavy capsule. No adhesions.

OPERATION, March 20

48. Right ovary about two-thirds removed, in addition to most of capsule. Stalk left intact. Some bleeding. Left ovary not removed.

Examined April 23 (5 weeks)

Right side. Ovary about average size. Capsule well defined. Large blood vessels spread over capsule.

OPERATION, March 20

49. Right ovary not removed. Left ovary about one-half removed, together with half of capsule. Stalk left intact. Some bleeding. (Ovary rather small.)

Examined April 23 (5 weeks)

Left side. Ovary normal in size. Capsule rather heavier than usual, and well supplied with blood vessels.

Examined August 10 (20 plus weeks)

Right ovary removed entire. Left ovary somewhat smaller than right, and two-lobed. Follicles evident.

OPERATION, March 20

50. Right ovary removed entire. (Ovary rather small.) Left ovary two-thirds removed, plus most of capsule. Stalk and mesentery left intact. Some bleeding.

Examined April 23 (5 weeks)

Right side: no evidence of ovarian tissue; tube normal. Left side: ovary average in size; capsule normal.

Examined August 10 (20 plus weeks)

Right side: no evident ovarian tissue; tube normal in size. Left side: normal sized ovary, in normal capsule.

OPERATION, December 31

55. Right ovary and capsule removed entire. Little bleeding. Left ovary incompletely removed; a small fragment left *in situ*.

Examined March 21 (11 weeks)

Right side: no evident ovarian tissue; tube normal in size and appearance. Left side: ovary had grown into an irregular mass of tissue, with a normal capsule; red.

While these operations were only roughly quantitative, nevertheless the regenerative capacity of ovarian tissue is illustrated in a striking manner. In four cases (47, 48, 49, 50) examination after only a 5-week interval revealed hypertrophy of the tissue left *in situ* into masses approximating or equaling in size the average normal ovary: one case after an 11-week interval showed the same condition; and two cases re-examined 20 plus weeks following operation (49, 50) gave evidence of no change from the condition at 5 weeks, which would suggest that such hypertrophy is of a permanent nature.

Since the completion of this work, a paper by Parkes and Bellerby (1926) has come to the writer's attention, in which support is lent to Davenport's findings. In connection with some work on hormone injection these workers conducted a series of ovariectomies on mice; and incidental mention is made of the fact that their results, so far as regeneration is concerned, "amply confirm" those of Davenport—the criterion of regeneration being the spontaneous onset of

the oestrous cycle. The probability of regeneration, indeed, was so great that they deemed it expedient to use castrated mice as test animals for only a short time following ovariectomy. They were able, however, to reduce the percentage of regeneration to a point below that found by Davenport by "removing the whole ovarian region, including capsule and fat body, at the time of operation." How material a reduction was obtained is not revealed, but the percentage of regeneration, presumably, still remained rather high. In their work, however, apparently no check was made as to the completeness of original removal, nor is their method of operation given; and it is the writer's opinion that their original operations were not complete in all cases.

SUMMARY

1. Germinal tissue in mice will not arise *de novo* from the coelomic epithelium following ovariectomy.

2. Regeneration of ovarian tissue in gonadectomized mice does not occur during a period of 32 weeks, providing all original germ-cell tissue has been removed; and it seems highly probable that it does not occur at all.

3. The occurrence of regenerated ovarian tissue in mice following ovariectomy can be satisfactorily explained on the basis of incomplete removal of the original ovary.

4. Following incomplete castration, regeneration occurs and growth of the regenerated material takes place very rapidly; that such regenerated tissue may be functional is indicated by the fact that follicles are sometimes present.

5. Such regeneration is of a permanent nature, and is not followed by absorption of the regenerated substance.

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The Respiratory Regulation of the Caryfish, *Cambarus immunis* (Hagen)

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THE RESPIRATORY REGULATION OF THE CRAYFISH, CAMBARUS IMMUNIS (HAGEN)

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INTRODUCTION

THE possible influence of the oxygen tension in the environment on the oxygen consumption of animals has interested many observers. The range of organisms studied has been considerable, and a brief survey of the types studied and results obtained is appropriate to the results described in this paper. Thunberg (1905), working on the air-breathing invertebrates (*Limax*, *Tenebrio*, *Lumbricus*), reported a partial dependence of oxygen consumption on the oxygen tension present in the environment. Henze (1910) in a comparative study of low and high invertebrates found that in the former (*Anemonia*, *Sipunculus*, *Actinia*) the dependence upon oxygen tension of the water was very marked in that oxygen consumption decreased as the oxygen tension was lowered. Conversely, the same author reports a relative independence of the oxygen consumption on the tension present for some of the higher invertebrates (*Eledone*, *Scyllarus*, *Carcinus*, *Aplysia*). On the basis of these findings, Henze drew the general conclusion that with the exception of some of the simpler organisms, the metabolic rate of cold-blooded animals is quite independent of the oxygen tension of their environment. This he interpreted as being due to the presence of adequate respiratory or circulatory systems whereby the oxygen tension of the various tissues remained unchanged. In the case of the lower invertebrates (*Anemonia*, *Sipunculus*, *Actinia*) the dependence on oxygen tension was explained as due to the lack of respiratory regulation, whereby the oxygen tension of some of the tissues is greatly reduced, perhaps even to zero. Krogh (1916) accepts, in general, Thunberg's explanation, believing that the lower invertebrates under question are provided with relatively imperfect respiratory systems and hence respiratory regulation under different

environmental oxygen tensions. McClendon (1917), working on *Cassiopea*, reports a lack of respiratory regulation in that lowering of oxygen tension of the water tends to lower the oxygen consumption. Lund (1921) likewise finds that in *Planaria* a dependence of oxygen consumption on the oxygen tension present is evident, although only after the oxygen tension has been reduced below the one-third saturation point of the water. *Paramecium*, on the other hand, according to the same author (1921), is quite independent of the oxygen tension present over a considerable range. More recently, Amberson, Mayerson, and Scott (1924–25) have shown that the oxygen consumption of some of the higher marine invertebrates (*Homarus americanus*, *Nereis virens*) is very closely dependent on the oxygen tension of the water over a rather wide range. In fact, the oxygen consumption at any point is directly proportional to the oxygen tension of the sea water at that instant. Preliminary experiments by the same workers give evidence that the same holds true for the king crab (*Limulus polyphemus*) and for the blue crab (*Callinectes sapidus*). The shrimp (*Palaemonetes*), however, was found to possess respiratory regulation down to about 50 per cent saturation; while the squid (*Loligo pealei*) was able to regulate its oxygen consumption down to about 30 per cent saturation. Below 50 per cent saturation, however, the squid (*Loligo pealei*) was found to lose its power of respiratory regulation, the amount of oxygen consumed being then proportional to the oxygen tension present. Amberson *et al.*, conclude, therefore, that Henze's distinction between the simpler and complex invertebrates as regards their dependence on the oxygen tension present cannot be regarded as absolute, since their data show a decided lack of respiratory regulation for certain complex invertebrates which possess well-developed respiratory systems. Their further observation that no general law can be made in this regard relative to the higher invertebrates is shared by the writer in view of the results to be described in the present paper.

It seemed profitable that a detailed study of the respiratory regulation in the crayfish be made in that the respiratory system, morphologically at least, resembles that of the lobster (*Homarus americanus*) very closely. The close resemblance of the two Crustaceans in this respect would suggest that respiratory regulation in

the two species would also closely approximate one another. The widely divergent environments, however, of the two species suggested the finding of a possible difference in degree of respiratory regulation. The results to be described indicate, in general, comparative independence of the oxygen tension present, coupled with an unusual degree of variation in oxygen consumption during consecutive hours when maintained under the same tensions. This latter fact has made inadvisable the grouping together and treatment of the data by statistical methods. Consideration of the animals as individuals, however, reveals certain distinct trends and facts which seem valuable in adding information toward the solution of the problems and generalizations as previously stated.

MATERIALS AND METHODS

The species of crayfish used for all tests made was that of *Cambarus immunis* (Hagen). They were collected as needed from a small pond near Iowa City, Iowa. When brought to the laboratory, the more healthy individuals were selected and placed in a balanced aquarium resembling as nearly as possible their natural environment. The animals were collected, in general, not more than four days before being tested. All collections were made during June and July.

The respiration of all individuals tested was based on their oxygen consumption per hour. In determining the latter, Birge and Juday's (1911) modification of the Winkler method was employed. The animals were in general tested singly. For this purpose rubber-stoppered $\frac{1}{2}$ liter, 1 liter, and 2 liter Erlenmeyer flasks provided with suitable inlet and outlet tubes were employed. The size of the animal determined the size of flask used. It was essential in this regard to place the animal in sufficient water so that the oxygen tension of the latter would not be lowered too rapidly. In this way the oxygen consumption over a wide range of tensions could be studied.

The tests were usually started at 8:00 A.M., and oxygen consumption determinations were made at the end of each hour thereafter, until the animals became asphyxiated or died. For the titrations, 30 cc. samples were drawn each hour. In no case did this constitute more than 6 per cent of the total quantity of water present

in the flasks. In all cases the water drawn was replaced by water of approximately the same tension. The oxygen content of the water added was determined for each addition, and the resulting total amount of oxygen present at the beginning of each hour could therefore be calculated. Some difficulty was experienced at first in securing water of low oxygen tension for addition to the flasks when the oxygen tension of the water in the latter dropped below 30 per cent saturation. In this connection it may be stated, parenthetically, that the crayfish under experiment were able to live under very low oxygen tensions and that the addition of but 30 cc. of slightly lowered oxygen tension water per hour contained enough oxygen to keep the animal alive for days. It was therefore necessary to add water of very low oxygen tension during these periods of the test. It was finally found that such water could be easily obtained by confining a dozen or more crayfish in a jar of water until they were asphyxiated. This water could then be diluted to any desired oxygen tension for addition to the flasks. Low-oxygen-tension water thus obtained seemed to have decided advantages over water deoxygenated by chemical means. The method was also more efficient than that of boiling the water.

In the group testing, ten crayfish were placed in a 5 gallon jar of water. The jars were fitted with outlet tubes and a glass stirring device for the thorough mixing of the water before samples were drawn. Because of the large initial quantity of oxygen present in these tests, it was not necessary to add water at the conclusion of the hourly tests, since the oxygen tension in the jars dropped very slowly. The decrease in the total oxygen available for the animals was determined for each hour, however, in order that the amount consumed per hour could be more accurately determined. The surface of the water in the jars was fully protected against diffusion of atmospheric oxygen by a 2 inch layer of petrolatum. Both the outlet tube and the stirring device passed through the petrolatum. As the animals became asphyxiated or died under low oxygen tensions, they were removed by means of a glass hook. This was found necessary since, as will be described later, asphyxiated and especially dead crayfish have a tendency to add oxygen to the water instead of removing it. Preliminary tests showed that the diffusion of atmos-

pheric oxygen through the petrolatum layer was negligible if not entirely inhibited.

Distilled water was employed throughout in order to obviate the possible ionic effect of chemicals normally present in tap water. In general the water used was approximately 100 per cent saturated at 25° C. partial pressure. The pH of such water averaged 6.7. All animals were starved for 48 hours before testing, and the testing flasks were immersed in water maintained at 25° C. No attempt was made to inhibit the activity of animals during the time they were confined to the flasks, since it was desirable to watch the effect of low oxygen tensions on such possible activity. As a matter of fact, however, the crayfish under experiment remained quiescent over long periods of time, sometimes for 5 or 6 hours without changing their position. Not infrequently the animal would move about for a few seconds. This seemed to become more prevalent as the oxygen tension was lowered. In a few cases the activity became excessive, and it was necessary to discard the data from such individuals. In general, activity was at a minimum and could not have seriously affected the oxygen consumption.

The animals were weighed to the tenth of a gram following the testing period, although this was not essential except as an indication of their size. No attempt was made to calculate the oxygen consumption per gram weight, since the tests were designed to determine the effect on the respiration of the animal as a whole under different oxygen tensions, the comparison of the metabolic rates between individuals of different weights and ages not being under consideration.

The samples of water drawn from the flasks were immediately titrated to avoid possible errors due to standing. N/300 thiosulphate was employed in place of the usual N/100 in order to lend greater accuracy to the tests. A Schellbach burette graduated to the 1/100 cc. was used in titrating, all titrations, whether by day or night, being read under a constant diffused light.

NORMAL VARIATION IN OXYGEN CONSUMPTION

Preliminary tests had shown that considerable variation existed in the oxygen consumption of animals when maintained under a

constant oxygen tension. It was thought advisable, therefore, to make a thorough study of such variation, in that such a factor must be considered in determining the possible regulation of respiration of animals during various oxygen tensions. Thirty individuals were therefore selected, ranging in weight from 3.0 grams to 28.2 grams, and their oxygen consumption determined, individually, for 5 consecutive hours. These tests were made in distilled water and under experimental conditions as previously described. The oxygen tension of the water at the beginning of the tests varied between 88 per cent and 116 per cent saturation at 25° C. partial pressure. For any one animal, however, the difference in the per cent saturation of the water between any two of the five consecutive tests did not exceed 3 per cent, which is a negligible amount considering the wide range of oxygen tensions investigated in connection with the main series to be discussed later. For the majority of animals, the variation between any two tests was but 1 or 2 per cent saturation. In the actual testing of the animals, the experimental flasks were emptied at the end of each hour and fresh distilled water from the same stock added for the next test. In this way the oxygen tension and purity of the water at the beginning of each hour was maintained quite uniform.

The data covering this series are represented in Table I. It will be noted that, in general, the variation in oxygen consumption is considerable between consecutive hours, although in a few cases (for example, animals 20, 28, and 30) the variation is quite small. Averages computed for the total amount of oxygen consumed by the entire group of thirty animals during each of the five consecutive hours reveal, perhaps, a better estimate of the variation present. The fifth-hour average in this regard is approximately 21 per cent lower than the first-hour average, although but 7 per cent lower than the third-hour average, the next lowest as compared with that of the first hour. It should be borne in mind, however, that the hour-by-hour variation of most individuals exceeded this per cent variation.

For the majority of animals tested (see Table I) the amount of oxygen consumed during the first hour was greater than that consumed during any of the following four hours. This was no doubt due to the fact that during the first-hour tests the animals were

placed in water at 25° C., a somewhat higher temperature than that of the stock aquaria in which the animals had been confined prior to testing. The oxygen consumption of ten crayfish, each tested in

TABLE I

NORMAL VARIATION IN OXYGEN CONSUMPTION OF CRAYFISH

ANIMAL	SEX	WEIGHT IN GRAMS	CENTIMETERS OF OXYGEN CONSUMED DURING CONSECUTIVE HOURS				
			First	Second	Third	Fourth	Fifth
1.....	♀	3.0	.755	.628	.510	.606	.507
2.....	♀	3.7	.547	.827	.758	.623	.804
3.....	♂	4.0	.497	.349	.448	.494	.383
4.....	♂	4.3	.788	.472	.434	.839	.632
5.....	♂	4.4	.763	1.059	.541	.516	.327
6.....	♀	4.7	.785	.414	.489	.779	.618
7.....	♂	4.8	.903	.504	.444	.891	.598
8.....	♀	4.8	.582	.346	.315	.425	.366
9.....	♂	5.2	1.402	.963	.878	.944	.668
10.....	♂	5.2	1.523	1.607	1.335	1.148	1.088
11.....	♀	5.3	1.162	1.068	1.027	.819	.959
12.....	♀	5.4	1.442	1.072	.827	.891	.895
13.....	♀	5.6	1.127	.812	.905	.748	.564
14.....	♀	5.9	1.015	.529	.365	.421	.588
15.....	♀	6.2	.954	.813	.773	.658	no test
16.....	♂	6.3	1.295	1.538	1.724	1.147	1.920
17.....	♂	6.5	1.227	.997	1.036	.843	.805
18.....	♀	6.5	1.202	.822	.692	.885	.822
19.....	♀	7.6	1.419	1.104	1.270	.871	.964
20.....	♀	7.8	1.863	1.948	1.990	1.875	1.924
21.....	♂	7.8	1.343	1.463	1.715	1.495	1.728
22.....	♂	9.4	1.721	1.075	1.530	1.382	no test
23.....	♂	9.5	1.629	1.234	1.862	1.310	no test
24.....	♀	13.3	2.326	1.510	1.273	1.295	1.524
25.....	♂	15.5	3.066	2.511	2.799	2.683	2.548
26.....	♂	15.5	1.774	1.627	1.504	1.519	1.233
27.....	♂	20.0	5.422	4.345	4.146	4.457	3.272
28.....	♂	20.0	1.991	2.097	2.044	1.932	2.224
29.....	♂	24.6	5.484	4.725	4.625	4.131	3.328
30.....	♂	28.2	4.796	4.591	4.845	4.814	4.614
Average..	.....	.....	1.693	1.435	1.437	1.381	1.330

water at aquarium temperature and at 25° C., confirm this conclusion. During the first hour, this rise in temperature, therefore, was responsible for a slight raising of the metabolic rate, which appears to have been only a temporary condition, since a common level, in general, was maintained during the following four testing periods.

There is no evidence to show that young crayfish exhibit a greater

amount of variation as compared with older animals, or vice versa. There is also no indication of a sexual difference regarding either the degree of variation present or the amount of oxygen consumed.

OXYGEN CONSUMPTION UNDER DIFFERENT OXYGEN TENSIONS

The complete data for all animals tested under various oxygen tensions is represented in Tables II–VI inclusive. The animals, as previously described, were tested in distilled water at 25° C. This

TABLE II

OXYGEN CONSUMPTION OF CRAYFISH AT VARIOUS OXYGEN TENSIONS

HOURS TESTED	ANIMAL									
	1 (1.5 Gm. ♀)		2 (1.7 Gm. ♀)		3 (3.0 Gm. ♀)		4 (4.3 Gm. ♂)		5 (4.5 Gm. ♀)	
	Per Cent Sat.*	Cc. O_2 Consumed	Per Cent Sat.	Cc. O_2 Consumed	Per Cent Sat.	Cc. O_2 Consumed	Per Cent Sat.	Cc. O_2 Consumed	Per Cent Sat.	Cc. O_2 Consumed
0	85	.....	85	.....	85	.....	85	.....	85	.....
1	79	.276	78	.208	73	.362	67	.598	61	.738
2	70	.097	67	.188	53	.425	43	.531	40	.521
3	59	.112	54	.221	36	.385	24	.497	22	.478
4	58	.107	46	.148	21	.367	10	.283	12	.270
5	41	.334	42	.032	13	.202	10	.129	10	.062
6	34	.141	29	.303	10	.094	8	.037	8	.035
7	28	.098	23	.078	9	.005	9	.015†	9	.013†
8	21	.170	19	.148	8	.046	8	.029	8	.031
9	19	.032	15	.092	8	.004	8	.007	8	.016
10	15	.096	13	.025	8	.016	8	.002	8	.004
11	13	.031‡	12	.025‡	7	.003‡	7	.027‡	8	.012‡

* The per cent saturation for hour 0 represents the oxygen tension of the water in the experimental flasks at the beginning of the test. The per cent saturation for all hours tested represents the oxygen tension of the water at the end of the hour.

† Indicates the addition of oxygen to the water by the animal, instead of oxygen being removed.

‡ Indicates asphyxiation or death of the animal during the hour.

water varied for different individuals between 85 and 132 per cent saturation, the exact per cent saturation used for each animal being indicated in the tables. Because of the fact that the animals reduced the oxygen tension of the water each hour to a different level by their respiration, it is difficult to group the data so that even four or five tests fall on the same oxygen tension. Furthermore, in cases where this occurred, the same four or five animals did not lower the oxygen tension during the following hours to exactly the same level. Consequently, it is necessary to consider (1) the individual behavior of

the animals undergoing successive hourly tests, and (2) the behavior of groups of individuals arranged according to their weights.

1. INDIVIDUAL BEHAVIOR UNDER VARIOUS OXYGEN TENSIONS

In general the oxygen consumption for the first hour was higher than that obtained for any of the succeeding hours, regardless of the oxygen tension of the water with which the test was started.

TABLE III

OXYGEN CONSUMPTION OF CRAYFISH AT VARIOUS OXYGEN TENSIONS

HOURS TESTED	ANIMAL									
	6 (6.3 Gm. ♀)		7 (6.5 Gm. ♂)		8 (7.5 Gm. ♂)		9 (7.9 Gm. ♀)		10 (8.1 Gm. ♂)	
	Per Cent Sat.*	Cc. O_2 Consumed	Per Cent Sat.	Cc. O_2 Consumed	Per Cent Sat.	Cc. O_2 Consumed	Per Cent Sat.	Cc. O_2 Consumed	Per Cent Sat.	Cc. O_2 Consumed
0	106	.....	106	.....	132	.....	132	.....	112	.....
1	94	.767	88	1.146	114	1.123	115	1.062	97	1.747
2	84	.565	76	.660	91	1.279	103	.602	80	1.984
3	75	.483	64	.753	68	1.344	87	.807	69	1.275
4	59	.968	56	.439	43	1.478	66	1.229	54	1.755
5	51	.515	48	.519	29	1.104	56	.533	45	1.251
6	41	.605	40	.540	21	.565	47	.608	35	1.394
7	33	.499	32	.589	22	.013	34	.802	27	1.100
8	.....	.....	.....	.....	20	.165	29	.301	19	1.015
9	.....	.....	.....	.....	17	.117‡	20	.599	15	.825
10	.....	.....	.....	.....	.....	.....	16	.293	19	.198†
11	.....	.....	.....	.....	.....	.....	19	.187†	15	.773
12	.....	.....	.....	.....	.....	.....	14	.377	9	.787
13	.....	.....	.....	.....	.....	.....	14	.011†	8	.273
14	.....	.....	.....	.....	.....	.....	20	.316†‡	8	.068
22	11	.415§	16	1.084§	.....	.....	.....	.....	.....	.....
23	.....	.....	.....	.....	.....	.....	.....	.....	13	.273†§

* The per cent saturation for hour 0 represents the oxygen tension of the water in the experimental flasks at the beginning of the test. The per cent saturation for all hours tested represents the oxygen tension of the water at the end of the hour.

† Indicates the addition of oxygen to the water by the animal, instead of oxygen being removed.

‡ Indicates asphyxiation or death of the animal during the hour.

§ Indicates that death occurred at some time since the last recorded test. The oxygen consumed is also for that period of time.

This fact can be attributed, as in the case of the results obtained for the normal variation in oxygen consumption, to the temperature of the water used in the experiments, as compared with the relatively cooler water of the stock aquaria. Following the first-hour test the oxygen consumption for successive hours underwent marked variation. In some cases relatively high and low amounts were consumed

on alternate hours. In others a steady decline in oxygen consumption occurred, only to be followed by a rise which sometimes became as high as the first few hours. In short, the variation in oxygen consumption during consecutive hours was of the same order as shown for the normal variation in oxygen consumption of animals tested

TABLE IV

OXYGEN CONSUMPTION OF CRAYFISH AT VARIOUS OXYGEN TENSIONS

HOURS TESTED	ANIMAL									
	11 (8.6 Gm. ♀)		12 (9.2 Gm. ♀)		13 (9.5 Gm. ♂)		14 (10.2 Gm. ♀)		15 (10.4 Gm. ♀)	
	Per Cent Sat.*	Cc. O_2 Consumed	Per Cent Sat.*	Cc. O_2 Consumed	Per Cent Sat.*	Cc. O_2 Consumed	Per Cent Sat.*	Cc. O_2 Consumed	Per Cent Sat.*	Cc. O_2 Consumed
0	100	.....	106	.....	132	.....	113	.....	106	.....
1	88	1.315	90	1.785	116	1.049	95	2.026	83	1.459
2	82	.800	74	1.938	103	.583	70	2.701	56	1.580
3	72	1.144	58	1.931	88	.820	47	2.471	30	1.573
4	61	1.443	46	1.540	76	.630	30	1.896	18	.745
5	52	1.163	33	1.682	60	1.161	19	1.157	12	.356
6	43	1.169	22	1.392	48	.595	14	.638	11	.087
7	43	.181	16	.842	34	.802	13	.138	12	.015†
8	32	1.660	12	.609	26	.476	14	.059†	11	.119
9	26	.717	12	.189	25	.132	12	.212	10	.100
10	24	.458	13	.054‡	21	.298	.....	.....	10	.022
11	14	1.462	.....	.....	20	.074	.....	.....	.....	.....
12	16	.050	.....	.....	20	.006	.....	.....	.....	.....
13	19	.112†	.....	.....	16	.271	.....	.....	.....	.....
14	15	.745	.....	.....	.....	.....	.....	.....	.....	.....
24	19	.170†§	.....	.....	.....	.....	10	.278§	.....	.....
29	.....	.....	.....	.....	26	.651†§	.....	.....	.....	.....

* The per cent saturation for hour 0 represents the oxygen tension of the water in the experimental flasks at the beginning of the test. The per cent saturation for all hours tested represents the oxygen tension of the water at the end of the hour.

† Indicates the addition of oxygen to the water by the animal, instead of oxygen being removed.

‡ Indicates asphyxiation or death of the animal during the hour.

§ Indicates that death occurred at some time since the last recorded test. The oxygen consumed is also for that period of time.

hourly in water of constant oxygen tension. This was true, in spite of the fact that the oxygen tension of the water in the experimental flasks steadily dropped as the animals consumed more and more oxygen. Eventually, however, there came a time when such regulation of respiration irrespective of oxygen tension ceased. The oxygen tension at which such regulation ceased was subject to variation depending on the animal. For a few, regulation ceased when the tension was lowered to approximately 35 per cent saturation. At the

lower extreme, regulation was maintained down to about 15 per cent saturation. The majority, however, were able to maintain their normal respiration down to between 25 and 19 per cent saturation. If the data for all the tests of the entire twenty animals tested be grouped together and the average oxygen consumption calculated

TABLE V

OXYGEN CONSUMPTION OF CRAYFISH AT VARIOUS OXYGEN TENSIONS

HOURS TESTED	ANIMAL									
	16 (12.0 Gm. ♀)		17 (14.9 Gm. ♂)		18 (16.4 Gm. ♀)		19 (18.5 Gm. ♀)		20 (18.7 Gm. ♀)	
	Per Cent Sat.*	Cc. O_2 Consumed	Per Cent Sat.	Cc. O_2 Consumed	Per Cent Sat.	Cc. O_2 Consumed	Per Cent Sat.	Cc. O_2 Consumed	Per Cent Sat.	Cc. O_2 Consumed
0.....	117	.....	122	.....	122	.....	127	.....	127	.....
1.....	99	2.111	92	3.405	82	4.598	92	4.013	100	3.185
2.....	82	1.241	56	4.015	50	3.678	63	3.167	71	3.207
3.....	73	1.394	33	2.504	25	2.764	34	3.290	43	3.131
4.....	58	1.529	22	1.197	17	.895	21	1.439	24	2.256
5.....	55	.271	17	.654	13	.461	14	.841	16	.827
6.....	38	1.883	14	.286	11	.234	13	.062	13	.413
7.....	30	.982	15	.122†‡	13	.142†‡	12	.159	14	.109†
8.....	21	.887	.....	.....	.....	.....	11	.099	9	.587‡
9.....	20	.120	.....	.....	.....	.....	10	.158	.....	.....
10.....	19	.190	.....	.....	.....	.....	8	.190	.....	.....
11.....	.....	.....	.....	.....	.....	.....	9	.047†	.....	.....
12.....	.....	.....	.....	.....	.....	.....	10	.126†	.....	.....
13.....	.....	.....	.....	.....	.....	.....	11	.043†	.....	.....
22.....	9	1.199§	.....	.....	.....	.....	.....	.....	.....	.....
23.....	.....	.....	.....	.....	.....	.....	11	.029†§	.....	.....

* The per cent saturation for hour 0 represents the oxygen tension of the water in the experimental flasks at the beginning of the test. The per cent saturation for all hours tested represents the oxygen tension of the water at the end of the hour.

† Indicates the addition of oxygen to the water by the animal, instead of oxygen being removed.

‡ Indicates asphyxiation or death of the animal during the hour.

§ Indicates that death occurred at some time since the last recorded test. The oxygen consumed is also for that period of time.

for each 10 per cent drop in oxygen tension, the average respiratory regulation appears to be in the neighborhood of 30 per cent saturation.

An interesting correlation may be drawn between the age of the animal, as indicated by his weight, and the range of respiratory regulation possessed. Animals 1, 2, 3, 4, 5, 8, and 9, ranging in weight from 1.5 grams to 7.9 grams and with an average weight of 4.3 grams, show regulation down to about 20 per cent saturation.

Animals 6, 7, 10, 11, 12, 13, 14, 15, and 16, ranging in weight from 6.3 grams to 12.0 grams and with an average weight of 9.0 grams, show regulation down to about 30 per cent saturation. Animals 17, 18, 19, and 20, ranging in weight from 14.9 grams to 18.7 grams and with an average weight of 17.1 grams, show regulation down to approximately 40 per cent saturation. This would seem to indicate clearly that the older the animal, the less the range of regulation, and vice versa.

Below the lower limit of the range of respiratory regulation, the oxygen consumption becomes very erratic and the animals are apparently unable to adjust themselves to the lowered oxygen tensions. The animals frequently become more active and swim to the top of the flasks in search of oxygen. Eventually, aphyxiation sets in, which process may be rapid or long drawn out, depending on the individual. For most individuals, however, the first signs of asphyxiation are discernible between the tensions of 15 per cent and 10 per cent saturation. The steps in asphyxiation are quite definite regardless of the size or sex of the animal. A general lack of vigor is first noted. the animal appearing to move about with difficulty. In some cases reddish cross-lines are discernible on the dorsal side of the abdomen between the segments, the exact nature of which was not determined. When movement finally ceases, the animals have just enough strength left to right themselves when turned on their backs. When this power is lost, they still continue to move their appendages feebly and the respiratory movements of the mouth parts cease entirely. In this condition they may lie for hours, consuming little or no oxygen. The last stage consists of the cessation of all movement, the animal appearing lifeless. If, however, the individual is placed in fresh water of high oxygen content, recovery occurs in the majority of cases. Death can generally be recognized by the curling up of the posterior segments of the abdomen. Complete asphyxiation usually occurred in water of 8–10 per cent saturation.

An interesting phenomenon occurred in most cases between the saturations of 20 per cent and 8 per cent. The animal appeared practically to cease respiration for an hour or more, and the tests for such periods indicated an addition of oxygen to the water, presumably by

the animal. This reversal of oxygen consumption was in general followed by respiration considerably higher in amount than experienced during the hours immediately preceding the cessation of the respiration period. Inhibition of respiration sometimes occurred again during the succeeding hours tested. Following the death of the animal, the body generally liberated oxygen enough to raise the oxygen tension of the water 1 per cent or 2 per cent. The possible explanation of this phenomenon will be discussed later.

2. GROUP TESTING UNDER VARIOUS OXYGEN TENSIONS

The testing of animals as regards their respiratory regulation in groups of ten was thought advisable in that it would serve to indicate directly the average behavior of the individuals concerned. The data for the three groups tested are represented in Table VI. The equipment used and method of group testing have already been described.

Group 1 consisted of ten crayfish ranging in weight from 16.0 grams to 19.2 grams and with an average weight of 17.3 grams. Group 2 consisted of ten crayfish ranging in weight from 11.5 grams to 23.6 grams and with an average weight of 18.0 grams; while the ten animals of Group 3, ranging in weight from 10.5 grams to 19.1 grams, had an average weight of 15.6 grams.

The general behavior and oxygen consumption of all three groups resembled, in general, the conditions found true for the individual testing of animals as previously described. The oxygen consumption with its corresponding drop in oxygen tension of the water was especially great during the first hour. During the next two or three hours the oxygen consumption varied according to the normal variation observed for most of the crayfish tested. The respiratory regulation for the three groups appeared normal down to approximately 40 per cent saturation. The average weight of the entire thirty animals tested amounted to 17.0 grams. Compared with animals 17, 18, 19, and 20, whose average weight was 17.1 grams, the range of respiratory regulation will be seen to be practically the same.

Respiration below 40 per cent saturation was likewise erratic, as previously described for the individual animal tests. The peculiar phenomenon of oxygen liberation by the animals during the lower oxygen tensions was especially prevalent. Since the crayfish were

removed from the testing jars as soon as complete asphyxiation occurred, the oxygen consumption for the following hours must be considered as due to the remaining individuals.

TABLE VI

COMBINED OXYGEN CONSUMPTION OF TEN CRAYFISH AT VARIOUS OXYGEN TENSIONS

HOURS TESTED	GROUP 1 (Av. Wt. 17.3 Gms. Ten ♀)		GROUP 2 (Av. Wt. 18.0 Gms. Ten ♀)		GROUP 3 (Av. Wt. 15.6 Gms. Seven ♀ Three ♂)	
	Per Cent Sat.*	Cc. O_2 Consumed	Per Cent Sat.	Cc. O_2 Consumed	Per Cent Sat.	Cc. O_2 Consumed
0	103	.....	102	.....	110	.....
1	76	30.100	69	36.192	77	36.367
2	47	32.172	47	23.860	58	21.094
3	37	10.856	34	14.784	42	17.749
4	21	16.998	22	13.154	26	17.353
5	18	3.760	15	7.121	17	9.328
6	14	3.618	16	.264†	17	.280
7	16	1.222†	13	3.058	13	5.086
8	12	4.144	12	1.115‡	14	.996†‡
9	12	.050	11	.578	11	3.179‡‡
10	12	.050	12	.483†	12	1.524†
11	12	.050	11	.686	11	1.082
12	11	1.307	11	.457	10	.775
13	10	.156‡	10	.585	12	1.713†
14	14	3.047†	10	.245	11	1.591†‡
15	15	.432†‡	10	.356‡‡‡	9	1.561‡
16	9	6.486	10	.275†‡	10	.665†
17	10	1.685†	9	.868‡	9	1.436
18	11	.985†‡‡	14	4.595†	10	1.064†
19	10	1.072‡‡‡	9	5.691	10	.147‡‡‡
20	10	.277	9	.166†	8	1.618‡
21	8	1.985	10	1.794†	11	2.647†
22	10	1.421†	9	1.141	10	.439
23	8	1.190	7	1.959	9	1.279‡
24	.....	.....	7	.233	.....	.....
25	.....	.....	5	1.893	.....	.....
26	.....	.....	10	4.114†‡	.....	.....
27	.....	.....	8	1.717‡	.....	.....
28	.....	.....	7	.665	.....	.....
29	.....	.....	9	1.883†‡	.....	.....
30	.....	.....	12	2.724†‡	.....	.....
31	8	.335§	.....	.....	.....	.....
32	8	.507‡	.....	.....	.....	.....

* The per cent saturation for hour 0 represents the oxygen tension of the water in the experimental jar at the beginning of the test. The per cent saturation for all hours tested represents the oxygen tension of the water at the end of the hour.

† Indicates the addition of oxygen to the water by the animals, instead of oxygen being removed.

‡ Each ‡ indicates the asphyxiation or death of an animal during the hour.

§ The § indicates the death of two animals at some time since the last recorded test. The oxygen consumed is also for that period of time.

The animals undergoing testing will be seen by reference to Table VI to exhibit widely varying degrees of resistance to asphyxiation. A few became asphyxiated when the oxygen tension dropped to from 14 per cent to 11 per cent saturation. The majority, however, were able to adapt themselves to the lower tensions so that asphyxiation did not occur until 5 to 8 hours later. Several remained in a weakened condition in various stages of asphyxiation for prolonged periods of time. In Group 2, for example, four animals resisted complete asphyxiation until the sixteenth, seventeenth, nineteenth, and twentieth hour, respectively, after the oxygen tension of the water had dropped to 12 per cent saturation. Three animals of Group 1 resisted asphyxiation until the twenty-third and twenty-fourth hours after the oxygen tension had reached 12 per cent saturation. In this latter group it was necessary to run the test for thirty-two hours before all animals were completely asphyxiated. The majority of the animals asphyxiated in the group testing recovered when placed in fresh water of high oxygen tension.

EFFECT OF H-ION CONCENTRATION ON OXYGEN CONSUMPTION

The respiration of the crayfish under analysis in the present paper would logically be expected to increase the hydrogen-ion concentration of the water in the experimental flasks to which they were confined. That this increase might prove to be of some magnitude likewise suggested itself as being plausible in view of the prolonged periods of time covered by the tests. Consequently, hydrogen-ion tests were made in connection with representative members of all oxygen consumption tests made. This included individuals of both sexes and of all sizes. Such tests revealed the fact that the greatest increase in hydrogen-ion concentration consisted in a drop in pH from 6.8 to 5.9. All other individuals tested increased the hydrogen-ion concentration to a pH intermediate between these two extremes. The natural inference suggested by this is that the increase in hydrogen-ion concentration might have influenced, in some manner, the oxygen consumption obtained for the individuals being tested. Consequently, an experiment was devised to determine the effect, if any, of hydrogen-ion concentration on the respiration of crayfish.

The technique employed in these tests was, briefly, as follows:

Five crayfish were selected of varying weights (see Table VII) and their oxygen consumption determined individually for six consecutive hours. The six consecutive tests were run in distilled water showing a pH at the beginning of the tests of 6.8, 6.0, 5.2, 6.7, 6.1, and 5.2, respectively. These different hydrogen-ion concentrations were obtained, artificially, by the diffusion of CO_2 through the water. The oxygen tension of the water was also the same at the beginning of each hour. The range of pH thus investigated was more than that exhibited by any of the animals of the oxygen-tension series. Moreover, the change of pH was far more rapid than in the latter series and involved a sudden raising of the pH between the third

TABLE VII

EFFECT OF H-ION CONCENTRATION ON OXYGEN CONSUMPTION OF CRAYFISH

ANIMAL	SEX	WT. IN GMS.	CONSECUTIVE HOURS											
			First		Second		Third		Fourth		Fifth		Sixth	
			pH	Cc. O_2 Consumed	pH	Cc. O_2 Consumed	pH	Cc. O_2 Consumed	pH	Cc. O_2 Consumed	pH	Cc. O_2 Consumed	pH	Cc. O_2 Consumed
1.....	♀	3.0	6.8	.354	6.0	.333	5.2	.416	6.7	.238	6.1	.330	5.2	.284
2.....	♀	5.1	6.8	1.179	6.0	1.130	5.2	1.190	6.7	.884	6.1	.841	5.2	.858
3.....	♀	8.2	6.8	1.421	6.0	1.307	5.2	1.624	6.7	1.262	6.1	1.321	5.2	1.207
4.....	♀	15.1	6.8	1.594	6.0	1.751	5.2	1.874	6.7	1.475	6.1	1.297	5.2	1.249
5.....	♀	18.2	6.8	2.113	6.0	1.359	5.2	2.071	6.7	1.914	6.1	2.026	5.2	1.836

and fourth hours which condition would tend to bring out any possible effect of hydrogen-ion concentration on oxygen consumption. This raising of the pH, however, never occurred in the oxygen-tension series, since the pH dropped steadily from the first hour of the tests to the last.

The data for the hydrogen-ion concentration tests are represented in Table VII. Reference to the oxygen consumed during each consecutive hour will reveal the fact that no marked effect was produced in any of the five animals tested. It is true that variation in oxygen consumption occurred, but such variations cannot be correlated with the change in pH or CO_2 content at which the tests were run. Furthermore, the variation present resembled that previously described and represented in Table I for the normal variation in oxygen consumption of animals tested for consecutive hours at ap-

proximately the same oxygen tension and pH. The conclusion to be drawn, therefore, is that within the range of pH and CO_2 content tested, changes in hydrogen-ion concentration and CO_2 content have no marked effect on the oxygen consumption of crayfish.

DISCUSSION

The normal variation in oxygen consumption is of such a magnitude in certain cases that oxygen consumption must be viewed as an unreliable measure of the metabolic rate for any particular hour. Fluctuations in the metabolic rate of invertebrates of such pronounced proportions are unknown to the writer. It seems more likely that some system whereby a reserve oxygen supply is maintained exists in the crayfish. When this reserve reaches a certain low point, as it is used up by the various tissues of the body, the external respiration of the animal then increases to build the reserve up to maximum. In this way a steady supply of oxygen would be distributed to the various tissues. It was noticed in a large number of cases that high oxygen consumption was correlated with increased activity of the mouth parts. Although the movement of the scaphognathites was maintained fairly constant from hour to hour, the movements of the mouth parts would govern to some extent the flow of water through the gill chambers.

A more logical and simpler explanation might be that the individual cells of the animal are capable, in themselves, of storing surplus oxygen which is then utilized at a certain rate in connection with their various metabolic activities until the supply is depleted. The reserve supply could then be thought of as being re-established. In this way the oxygen tension of the blood would be subject to some variation, and the attempt on the part of the animal to maintain it at a constant level would result in a variation of the amount of oxygen consumed. It is interesting to mention in this connection the peculiar power of crayfish to live for some time when removed from the water. Whether or not atmospheric oxygen is utilized at this time or oxygen is derived from an internal reserve supply has not been determined. The problem is an interesting one, however, and warrants investigation.

The data covering the range of respiratory regulation of indi-

viduals under different oxygen tensions indicate quite clearly that crayfish exhibit respiratory regulation over a considerable range. This is in direct contrast to Amberson, Mayerson, and Scott's (1924–25) work on the lobster, where oxygen consumption was found to be proportional to the oxygen tension of the sea water. The contrast is interesting in that both organisms possess practically the same respiratory system, structurally. Such a contrast seems plausible to the writer in view of the wide differences in physiological properties between sea water and distilled water. Furthermore, there is no logical reason why two structurally similar respiratory systems should function similarly in all respects.

The erratic respiration of crayfish below the lower limit of respiratory regulation suggests, at least, that physiological regulation of respiration can no longer be maintained in a normal manner and hence is followed shortly by disturbances leading to the asphyxiation of the animal. That certain individuals are able to resist the onset of asphyxiation for some time appears evident from the data presented. Evidently they possess a greater degree of adaptability in this regard. This would seem especially applicable to the species of crayfish investigated, in that crayfish living in ponds are normally subject to considerable variations in the oxygen tension of the water, the latter being due to temperature changes and reduced volumes during droughts. The writer has kept one crayfish in the laboratory for over three months in a small jar of water whose oxygen tension averaged 26 per cent saturation at room temperature. It seems possible, therefore, that pond and sluggish-water crayfish possess a certain degree of adaptability to lowered oxygen tensions. Whether or not this adaptability is shared by crayfish living in swift-running streams has not been determined. The inference, however, is that such forms, not being normally subjected to great variations in oxygen tension, would not possess so great a degree of adaptability in this respect.

The fact that older crayfish appear unable to regulate respiration under as low an oxygen tension as young animals presents a situation difficult of explanation. Certainly, the completeness of the structural development of the respiratory system cannot be used here as an explanation, since the older animals must possess a

respiratory system as fully developed as, if not more fully developed than, that of the young animals. It can only be suggested here that the body cells of older animals may have lost in some way the physiological adaptability of the body cells possessed by young animals toward low oxygen tensions.

The phenomenon presented by many of the tested animals whereby an apparent reversal of oxygen consumption occurs when under low oxygen tensions is likewise difficult of adequate explanation. The fact that such liberation of oxygen by the animal took place only during comparatively low oxygen tensions would again suggest the possibility of a temporary loss in the power of the body cells to retain a reserve supply of oxygen in a normal manner. It seems possible that under lowered tensions the oxygen reserve may be suddenly released. The fact that during the following hour or hours oxygen was in general consumed again by the animals indicates a partial recovery of oxygen retention. In general, it will be noted, however, that such reversals in oxygen consumption became more frequent after having first occurred, and were especially prevalent following the onset of asphyxiation.

That changes in the hydrogen-ion concentration and CO_2 content of the water may have influenced the rate of oxygen consumption was shown to be without foundation in view of the tests made concerning this point. For all practical purposes, the variation in oxygen consumption during changing hydrogen-ion concentrations and CO_2 content might have been employed in representing the normal variations in oxygen consumption of animals in water of a constant pH and CO_2 content. This finding coincides with Amberson, Mayerson, and Scott's (1924–25) results on the lobster, where changes in the CO_2 tension of the sea water, much exceeding a range of pH 8.1–7.6, were found to have no effect on the rate of oxygen consumption.

During the course of the work described in the present paper, eight cases of molting were observed which occurred during the testing periods. Although data from such animals were discarded, it was noted that no marked changes in oxygen consumption occurred which might be attributed to this process. Five of these cases survived the process, possibly because of the fact that molting occurred

when the water in the experimental flasks was still at a relatively high oxygen tension. The remaining three died shortly after or during the process. The oxygen tension of the water at which these three molted was relatively much lower than in the case of the five surviving the molting process, which fact possibly accounted for their death.

SUMMARY AND CONCLUSIONS

1. Thirty crayfish (*Cambarus immunis*) were tested for five consecutive hours using a modified Winkler method for determining oxygen consumption. The hourly variation in oxygen consumption was found. in general, to be considerable, although the tests were made with distilled water at a constant temperature, oxygen tension, and hydrogen-ion concentration. Attention is called to the fact that such variation must be considered in the analysis of oxygen consumption under various oxygen tensions.

2. The oxygen consumption of twenty crayfish was determined, individually, for consecutive hours in water undergoing a decrease in oxygen tension resulting from the metabolism of the animals. Regulation of respiration was found to be present down to 20 per cent saturation for animals averaging 4.3 grams in weight, down to 30 per cent saturation for animals averaging 9.0 grams in weight, and down to 40 per cent saturation for animals averaging 17.1 grams in weight. The conclusion is drawn that the older the animal, the less the range of respiratory regulation.

3. Below the lower limit of the range of respiratory regulation, oxygen consumption becomes erratic. Cases are cited where an apparent reversal in oxygen consumption occurs, the animals liberating oxygen to the water at low oxygen tensions. Asphyxiation occurs at low tensions in definite stages and may be rapid or considerably extended.

4. Thirty crayfish were tested in groups of ten each to determine the effect on oxygen consumption under decreasing oxygen tensions. The results were similar to those obtained with crayfish tested individually having the same weight.

5. Five animals were tested for six consecutive hours in water having hydrogen-ion concentrations of pH 6.8, 6.0, 5.2, 6.7, 6.1, and 5.2, respectively. No marked effect on the oxygen consumption due

to changes in hydrogen-ion concentration and CO_2 content were recorded. The conclusion is drawn that within the pH range investigated, hydrogen-ion concentration has no effect on oxygen consumption.

6. Suggestions and possible explanations are offered to explain the normal variation in oxygen consumption, the decrease in range of respiratory regulation for older animals, and the apparent paradox of reversal of oxygen consumption during low oxygen tensions and asphyxiation.

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3904

Relief of Parathyroid Tetany by Injections of Uranium Nitrate.

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Recent studies from this laboratory[1, 2] have shown that slight changes in the acid-base equilibrium, with a shift in the reaction of the blood toward the acid side, exert a profound effect upon the tetany of parathyroid insufficiency.

The writer and his collaborators have shown that asphyxiation by CO_2 of dogs presenting violent tetany promptly brings about a return to normal in so far as tetany symptoms are concerned. The disappearance of symptoms was correlated with disturbance of the acid-base equilibrium, *i. e.,* lowered CO_2 capacity, CO_2 content and pH, due to rise in lactic acid. Tetany again supervened following a return to a normal acid-base balance and disappearance of the lactic acid. The total serum calcium remained unchanged by the CO_2 treatment, despite the fact the animals seemed normal and were free from tetany.

Since the effect of the CO_2 lasts but a brief period, and since it was considered desirable to study the experimental animals over longer periods, the present experiment with uranium nitrate was undertaken. This substance has been extensively employed in the study of renal function since it induces marked pathological changes in the kidneys when injected, and it is a well established fact that a profound acid intoxication develops in animals so treated. It is not definitely known to what acid or acids the acidosis of uranium nitrate poisoning is due. The intoxication develops slowly and reaches a maximum between the 4th and 8th day following injection.

In the present experiments both old and young dogs were used. They were thyroparathyroidectomized and allowed to develop marked tetany symptoms. They were then bled for CO_2 capacity,

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3906

I. Effect of Placental Extract on Mammary Glands of Male Guinea Pigs.*

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Although considerable work has been centered upon the influence of placental hormone upon secondary sexual characters in the female, particularly the rabbit and the guinea pig, little attention has been directed toward its possible effects upon those of the male. Hermann[1] states briefly that he induced development of the mammary glands in the young male rabbit, through injection of the "active substance" of corpus luteum and of placenta, even to the extent of producing milk secretion, and he concludes that this activating substance is of powerful influence in the formation of specific sex characters. No evidence is offered, however, in support of his statements, nor are any particulars of the experiments given. Fellner[2] reports in somewhat more detail 2 cases of a similar nature in young rabbits, in which a slight degree of hypertrophy was induced but, unlike Herrmann, he was unable to produce a secretion of milk.

In connection with some work with the placental hormone, 4 adult guinea pigs were castrated and injected twice daily with ½ cc. doses of human placental extract (furnished through the courtesy of Parke Davis & Co., Detroit, under the trade name of "Estrogen"): the total daily dosage amounting to 20-25 rat units. Three control castrates were given equal amounts of normal saline.

After 8 days of extract injection, a swelling of the mammary regions became noticeable; the nipples increased in size and the surrounding areolar areas assumed a turgescent appearance. This hypertrophy continued steadily over a period of some 3 weeks, after

* The expenses of this investigation have been defrayed by a grant from the Committee on Research in Problems of Sex of the National Research Council.

which time continued injections produced no further effect. The hypertrophied glands, together with controls, were removed, photographed *in toto,* and sections were made of nipples and of glandular tissue. In the injected animals the nipples displayed a marked hypertrophy, evidenced particularly by the epithelium and by a thickening of the stratum corneum, there being an increase to approximately 3 times that of the controls. The glandular structures, extremely small and scanty in the untreated male, increased in proportion through a decided growth and branching of ducts, through growth of the alveoli, and through widening of the lumina. This held true also for the lobules, although there appeared to be no increase in their actual number. There was evidence of active secretion as indicated by the presence of colostrum corpuscles and what appears to be milk.

[1] Herrmann, E., *Monatsschr. f. Geburtsh. u. Gynak.,* 1915, xli. 1.

[2] Fellner, O., *Arch. f. Gynak.,* 1913, c, 641.

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A STUDY OF THE SURVIVAL PERIOD IN THE PREGNANT AND LACTATING CAT FOLLOWING BILATERAL ADRENAL EXTIRPATION[1]

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H. A. STEWART (1913) first called attention to what he considered to be an unusual prolongation of the life-span of the pregnant cat following adrenal removal. He states: "In most instances the pregnant and lactating animals survived the extirpation of the adrenal glands longer than normal, non-pregnant or male animals." However, his average prolongation in good cases of non-injected pregnant females was only 2.2 days.

In 1925, G. M. Stewart and Rogoff working with dogs cited cases of pregnant bitches which lived for considerable periods following bilateral adrenal removal. Several animals survived throughout the gestation period, and one animal gave birth to a litter of pups. These authors do not attempt to explain the reason for such prolongation of the survival period of adrenalectomized dogs, but merely suggest that possibly the corpus luteum may be involved.

In an earlier series of experiments on bilaterally adrenalectomized cats (Corey, 1926), the writer observed that pregnant individuals did not survive any longer than male or non-pregnant animals when the fact is taken into consideration that the average life-span of a double operated cat is 100 hours or more. The observations at that time were limited to only a few cases, hence no significance was attached to them. During the spring of 1927, however, the problem of the survival period of double-operated pregnant cats was carefully studied. A series of sixteen female animals was operated and their survival periods were checked against those of six operated male and

[1] A part of the expense of this investigation was defrayed by a grant from the Committee for Research in Problems of Sex of the National Research Council. Grant administered by W. W. Swingle.

non-pregnant female animals which served as controls for this particular experiment.

This problem was suggested by Dr. W. W. Swingle, of the Zoölogical Laboratory of the State University of Iowa, formerly of this laboratory, and carried out under his direction. Grateful acknowledgments are made to Dr. R. G. Harrison for laboratory facilities and the opportunity to continue the work at Yale.

All animals were operated with a 3–17 day interval between the removal of the right and the left adrenal glands. They were kept at a temperature of approximately 22° C. and were permitted the freedom of the animal room. Food was at all times available to them but no attempt was made to feed them forcibly[1] following the onset of anorexia which usually succeeds (40–48 hours) the removal of the glands. The animals were examined daily for infection but none was observed. All animals eventually died in coma, following the typical syndrome attendant upon the extirpation of both adrenals. At death each animal was carefully autopsied and histological sections were made of the ovaries in those cases in which any doubt existed as to whether an intervening ovarian cycle had taken place since abortion or resorption, where such was indicated by the condition of the uterine walls. A careful search was made at every autopsy for macroscopic pieces of cortical tissue, as is necessary in all survival experiments of this nature. No such cases have been recorded in this paper, since they are valueless for the present study.

The short survival period for animals 1, 2, and 6, as recorded in the table, is hard to explain. The fetuses found in the uterus of No. 1 (four in number) were apparently at term, or at any rate very closely approximating that stage. They were normal in all respects. The fact that the animal died tends, in the opinion of the author, to support the view of Stewart and Rogoff—that the presence of the adrenal cortex of the fetus has little or no ameliorative effect upon the symptoms of the mother.

The fetuses (four in number) found upon the autopsy of animal No. 2 were normal and somewhat over two weeks of age.

[1] Exceptions were made in the cases of Nos. 5, 6, and 19, to which 30 c.c. of milk were administered twice daily, by means of a stomach tube. No increase in the survival period due to this treatment is indicated.

Animals 3, 4, and 5 show a survival period approximately identical with that of the male control animals.

In the case of the animal in very early pregnancy (No. 7), the developing embryonic vesicle was clearly identified. The walls of the

TABLE I

No.	Sex	Days Elapsed between First and Second Operation	Survival after Second Operation (hours)	Remarks	Survival Average (hours)
1...	Female	7	49.0	Four term fetuses in uterus at autopsy	86.0 (116.5 for Nos. 3, 4, and 5)
2...	Female	5	69.5	Four two-week fetuses in uterus at autopsy	
3...	Female	5	140.0	Four 1½-in. fetuses in uterus at autopsy	
4...	Female	8	110.0	Four 1-in. fetuses in uterus at autopsy	
5...	Female	17	99.5	Three fetuses in uterus at autopsy averaging 1.4 cm. in length	
6...	Female	5	48.0	Five 9.0 cm. fetuses in uterus at autopsy	
7...	Female	5	210.0	Very early pregnancy	
8...	Female	5	242.0	Aborted four fetuses 48 hours after operation (about ½ term.)	180.0
9...	Female	5	120.0	Aborted three 2-in. fetuses 36 hours after operation	
10...	Female	6	97.0	Gave birth to three kittens 16 hours after operation. Suckled young for 24 hours	
11...	Female	7	69.5	Gave birth to four kittens 8 days before final operation. Suckled young 24 hours, four corpora lutea on ovaries	
12...	Female	7	216.0	Resorption of three fetuses	
13...	Female	7	141.0	Non-pregnant	110.0
14...	Female	5	71.0	Non-pregnant	
15...	Female	7	119.0	Non-pregnant	
16...	Male	5	95.0	Control	127.3
17...	Male	5	118.0	Control	
18...	Male	5	169.0	Control	
19...	Female	3	42.0	Pregnant (?) Four corpora lutea found on ovary at autopsy	

uterus were considerable distended and the placental formation normal.

Animal No. 8 aborted four fetuses 48 hours after the second operation. They were apparently at about half-term. (Since in no case was the time of insemination recorded, the age of all fetuses is necessarily a rough estimate.) Upon autopsy, 194 hours later, one almost completely resorbed fetus was found in the uterus. The walls of this organ were distended, the remains of the placenta were of a typical dark-brown coloration, and the uterine vessels were greatly enlarged.

Animal No. 9 aborted three two-inch fetuses 36 hours following the second operation. It, however, lived for 120 hours after the second operation, dying with typical symptoms of adrenal insufficiency.

No. 10 gave birth to three full-term kittens 16 hours after the second operation. The young were suckled for 24 hours, after which the mother paid them no further notice. She died 97 hours after the removal of the second gland. At autopsy the uterus was found to be empty.

No. 11 gave birth to four kittens (two dead) eight days before the removal of the second gland. The kittens were suckled for approximately 24 hours following complete epinephrectomy. The mother died 69.5 hours following the second operation.

Animal No. 12 showed practically complete resorption of three fetuses. The condition of the uterus indicated quite clearly that no ovarian cycle had succeeded the pregnancy. The short time-interval from operation until death makes a case of complete resorption somewhat dubious. It can only be looked upon as a case of very early vesicle formation, followed by a succeeding rapid resorption. The walls of the uterus were quite markedly distended and the placenta of a characteristic brownish color. The uterine vessels were greatly distended.

The non-pregnant female animals were employed as control animals, their survival period being approximately identical with that of the male controls. As pointed out in a previous paper (Corey, 1927), a survival period of approximately 100 hours, or over, must be looked upon as the normal life-span for bilaterally epinephrectomized

cats. However, if fluids are forcibly fed or are injected, the life-span is considerably prolonged (Corey, 1926.)

Animal No. 19 is a case of doubtful early pregnancy. No fetuses were found in the uterus at the time of autopsy, but four corpora lutea were clearly made out on the surface of the ovaries. The uterine vessels were somewhat distended.

As will be seen from the accompanying table, the pregnant animals (with the exception of Nos. 7, 8, and 12) show a survival period closely approximating that of the control animals. The fact that none of the pregnant animals (with the exception of No. 10) lived until the delivery of its litter indicates that whatever mechanism may be present in the dog which enables the epinephrectomized mother to live until the culmination of the pregnancy, this mechanism is apparently lacking in the cat. Since No. 10 delivered its litter only 16 hours after the second operation, it can hardly be taken as a case controverting the foregoing statement.

Animals 7, 8, and 12 survived considerably longer than the average period for adrenalectomized cats but not longer than double-operated cats treated by forcing fluids. As shown in the table, animals 7 and 12 were cases of very early pregnancy, whereas No. 8 was a case of advanced pregnancy. The longest survival period for a pregnant animal was 242 hours, whereas the longest survival period for a non-pregnant cat was 169 hours. The writer is not inclined to lay too much stress upon these cases since they all died within 10 days following the second operation.

It is interesting to note that cats and the dogs react differently to adrenal insufficiency in several respects. For example, adrenalectomized dogs usually show gastro-intestinal disturbances such as vomiting; they also are subject to yelling fits and hallucinations, and upon autopsy show great congestion and extravasation of blood into the gastro-intestinal tract. Operated cats do not present these symptoms, or if so are exceptions to the general rule. So, too, with regard to the effect of pregnancy and lactation upon the survival period following adrenal removal. In the dog the life-span following adrenalectomy is apparently greatly prolonged by pregnancy (Stewart, G. M., and Rogoff), but such appears not to be the case with cats.

CONCLUSIONS

1. The presence of the adrenal cortex of the fetal cat has little or no ameliorative effect upon the syndrome of the epinephrectomized mother.

2. The survival period of bilaterally epinephrectomized pregnant cats is approximately the same as that of male and non-pregnant female animals, similarly operated.

3. Whatever mechanism is present in the dog enabling the epinephrectomized mother to live until the culmination of the pregnancy, this mechanism is apparently lacking in the cat.

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The Effect of CO_2 Administration upon Parathyroid Tetany

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THE EFFECT OF CO_2 ADMINISTRATION UPON PARATHYROID TETANY

(Four figures)

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IT HAS been known for some years that certain mineral acids such as HCl and acid-producing salts such as $Ca\ Cl_2$ and NH_4-Cl are effective agents in the treatment of infantile tetany, and have also been successfully employed in cases of experimental tetany.

Wilson, Stearns, and Thurlow (1915) in a series of experiments performed upon parathyroidectomized dogs observed that injection of 0.1 to 0.5 grams of HCl relieved the tetany symptoms.

György reported (1922) that the administration of such salts as calcium chloride and ammonium chloride gave excellent results in cases of infantile tetany. This has been confirmed by Gamble and Ross (1925) and Anderson and Graham (1924).

Wenner (1927) tested the effect of NH_4Cl on the prevention of experimental tetany in dogs, and the action of this acid-producing salt on the serum calcium level of parathyroidectomized animals. He noted that NH_4Cl is efficacious in preventing the onset of tetany in operated dogs, and when administered orally in large doses to animals presenting severe symptoms and low serum calcium, it alleviates the symptoms and induces a rise in the serum calcium.

Wenner and Muntwyler (1927) observed during the course of their study of alkalosis and tetany several cases of apparently complete recovery from violent tetany convulsions, the improved condition lasting several hours. Study of the blood chemistry of these cases revealed that the pH and alkali reserve of the blood fall far below the normal—pH 7.09, CO_2 content 26.8 vol. per cent in one case.

The effect of injecting HCl and $CaCl_2$ or NH_4Cl is the production of an acidosis. For instance Haldane, Hill, and Luck (1923)

clearly demonstrated that a change in the reaction of the blood toward the acid side follows ingestion of large amounts of $CaCl_2$. The Ca is eliminated through the alimentary tract as calcium carbonate, whereas the chloride is almost completely absorbed and replaces the bicarbonate of the blood thus inducing acid intoxication.

It seemed evident from a survey of the literature on the subject,

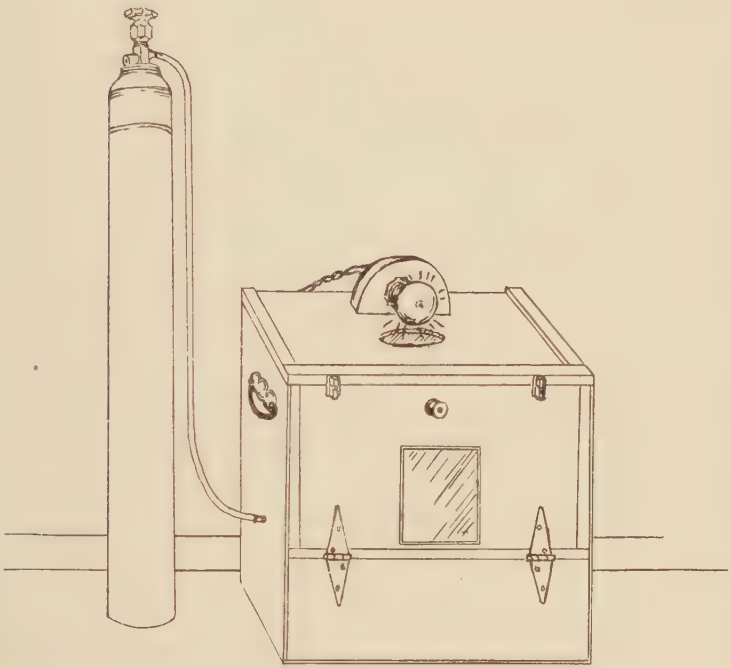

FIG. 1.—CO_2 chamber used in the experiments

that the gross manifestations of tetany—even the most violent convulsions—are rather promptly relieved by slight changes in the reaction of the blood toward the acid side, and that such changes influence in some manner the calcium content of the blood. In view of these facts it was considered worth while to test the effect of administering CO_2 to dogs in tetany (1) in relation to relief of symptoms, and (2) the effect upon the serum calcium level.

The method of CO_2 administration was simple and is illustrated by the schematic drawing of the apparatus employed (Fig. 1). A

specially constructed air-tight chamber was used, the only special feature consisting of a thick glass panel in the front through which the respiration of the animal could be followed, and at the top a small oval glass with an electric light bulb and reflector just above and outside the chamber. No effort was made to determine the exact percentage of gas in the chamber. We were interested chiefly in the effects of asphyxiation upon the tetany syndrome and not in the effect of CO_2 per se, and used this gas chiefly because of the ease with which it lent itself to our purpose. It is highly probably that nitrogen or any inert gas would have served as well as CO_2.

Small rat and fox terriers were used as experimental animals. They were thyroparathyroidectomized and permitted to develop tetany. When the animals were prostrate and in a condition similar to that of the animal shown in Figure 2, they were bled and the serum Ca determined by Clark and Collip's (1925) modification of the Kramer-Tisdall method (1921). Following bleeding the dogs were immediately placed in the CO_2 chamber and sufficient gas administered to produce unconsciousness. We observed that slow intermittent administration of the gas during an interval of 20-40 minutes until unconsciousness supervened is more effective in so far as the relief of tetany symptoms is concerned than very rapid asphyxiation with large quantities of CO_2. The animals require constant watching while in the box and must be removed immediately after losing consciousness or else it is impossible to revive them. The second blood was obtained by cardiac puncture while the animals were reviving or very shortly after they regained consciousness.

The rapidity with which the muscle tremors, jerks, and convulsions disappear during asphyxiation is remarkable. Within two minutes following entrance of the gas into the chamber manifest tetany disappears. The struggles of the slowly asphyxiating animal are of a quite different nature from tetany and the difference is obvious to even the casual observer. Our best cases were dogs which developed violent tetany during the day and which had not been prostrate for hours. Our poorest cases in so far as recovery from tetany after asphyxiation was concerned were those dogs in which violent attacks of tetany occurred during the night and the animals were exhausted when placed in the CO_2 chamber. Such dogs do not react

in such a striking manner as the cases exhibiting tetany but a short time.

The animals very quickly recover when removed from the CO_2 chamber and exhibit no symptoms of tetany. They run about, play, eat, and behave as do normal unoperated dogs. One of our cases engaged in a hearty fight with another dog shortly after removal from the box, whereas but a short time previously he had been prostrate with tetany.

The length of the recovery period varies considerably with the various animals. Some dogs do not again exhibit tetany symptoms for twelve hours or more, others show tremors and even more violent symptoms within a few hours. If such animals are again placed in the CO_2 chamber and gas administered, they again temporarily recover. No effort was made to keep the animals alive indefinitely by the experimental procedures employed, since we were primarily interested in the effect of acid intoxication upon the serum calcium and the relief from tetany.

Figure 2 shows a dog prostrate in violent tetany spasms a few moments prior to placing it in the CO_2 chamber. Figure 3 shows the same animal one hour after a twenty-minute exposure to CO_2. Figure 4 shows the dog twelve hours later. All of the animals used in the experiment died of tetany.

In Table I is shown the effect of the CO_2 treatment upon the level of the serum calcium, and the changes in the CO_2 content, CO_2 capacity, and pH of the blood a few minutes after removal from the chamber. Determinations of the serum calcium made later (1-4 hours) after asphyxiation by CO_2 showed no changes in the serum calcium, but a return to approximately normal in so far as the CO_2 content, CO_2 capacity and pH of the blood were concerned.

It is quite evident from the figures given in Table I that the dogs were suffering from a marked acid intoxication when removed from the CO_2 chamber. The changes in the CO_2 capacity, content, and pH of the blood clearly indicate acidosis. It is also obvious that the acidosis present was not due to accumulation of CO_2, for the CO_2 capacity, and content of the blood was invariably low and this holds true for the pH also. The results indicated that some organic acid was probably responsible for the observed blood changes. Since the

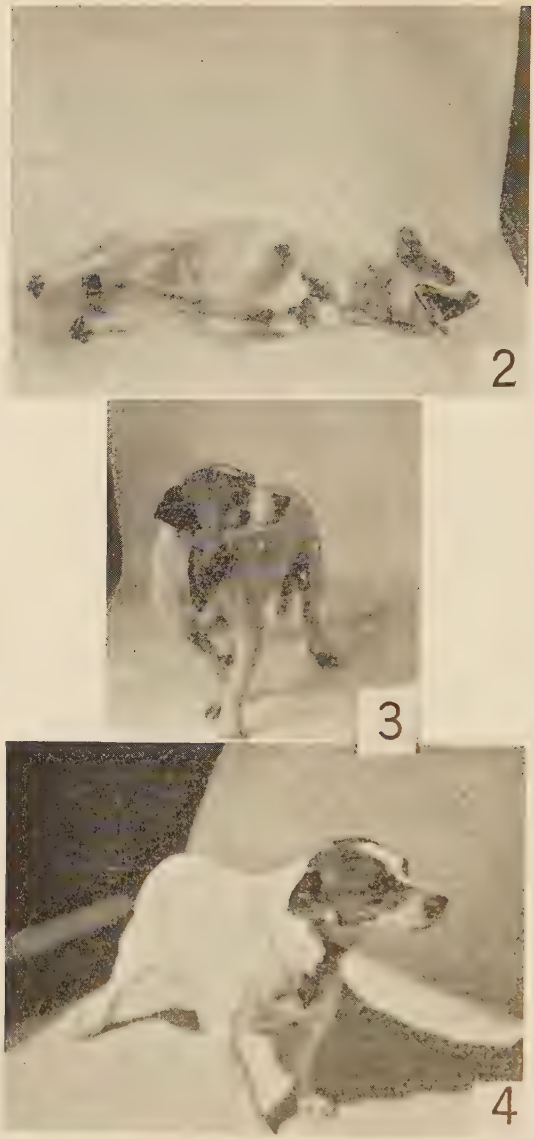

FIG. 2.—Dog in violent tetany before CO_2 administration
FIG. 3.—Same dog as shown in Figure 2 after CO_2 treatment
FIG. 4.—Same animal twelve hours after treatment

TABLE I

Animal	Tetany Dogs before CO_2					Tetany Dogs after CO_2					
	Symptoms	Mgm per 100 c.c. Ca	CO_2 Capacity	CO_2 Content	pH	Symptoms	Mgm per 100 c.c. Ca.	CO_2 Capacity	CO_2 Content	pH	Remarks
1.....	Violent tetany	5.9	.....	.....	.....	None	6.	.....	.....	.....	CO_2 given for 20 min.
2.....	Violent tetany	6.7	.....	.....	.....	None	7.2	.....	.....	.....	CO_2 given for 1½ hrs.
3.....	Violent tetany	6.4	.....	.....	.....	None	7.	.....	.....	.....	CO_2 given for 32 min.
4.....	Violent tetany	6.5	.....	.....	.....	None	7.6	.....	.....	.....	CO_2 given for 26 min.
5.....	Violent tetany	5.9	.....	.....	.....	None	6.	.....	.....	.....	CO_2 given for 19 min.
6.....	Violent tetany	6.4	.....	.....	.....	None	7.	.....	.....	.....	CO_2 given for 45 min.
7.....	Violent tetany	6.8	.....	.....	.....	None	7.1	.....	.....	.....	CO_2 given for 23 min.
8.....	Violent tetany	5.7	.....	.....	.....	None	5.8	.....	.....	.....	CO_2 given for 20 min.
9.....	Violent tetany	6.3	45.6	42.1	7.38	None	6.8	32.1	28.1	7.21	Unconscious when taken from chamber
10.....	Violent tetany	6.7	47.3	40.6	7.39	None	7.	28.	24.	7.22	CO_2 given for 20 min.
11.....	Violent tetany	6.4	48.2	43.1	7.4	None	7.	30.1	27.2	7.25	CO_2 given for 25 min.
12.....	Violent tetany	6.5	56.	48.	7.36	None	7.6	31.1	28.	7.14	Unconscious when taken from chamber
13.....	Violent tetany	6.5	50.5	43.7	7.46	None	6.7	36.	32.2	7.28	CO_2 given for 24 min.
14.....	Violent tetany	6.	45.7	38.1	7.36	None	6.	28.7	26.2	7.14	CO_2 given for 45 min.
15.....	Violent tetany	6.5	54.	45.	7.38	None	7.	33.	30.	7.22	CO_2 given for 45 min.
16.....	Violent tetany	6.2	48.7	40.8	7.44	None	6.6	30.9	28.7	7.19	Unconscious when taken from chamber

CO_2 treatment was really a simple method of asphyxiation, it seemed probable that lactic acid was the responsible agent. The idea seemed valid in view of McGinty and Gesell's (1925) work on the effect of CO administration upon normal dogs. They observed that asphyxiation by CO causes a striking increase in the lactic acid content of the blood. Their figures for the normal lactic acid of dog blood was 23.9 mgm. per 100 c.c. whereas following CO administration the lactic acid rose to 75.1 mgm. per 100 c.c.

TABLE II

Animal	Time	Lactic Acid Mgm. Per Cent	CO_2 Content Vol. Per Cent	CO_2 Capacity Vol. Per Cent	pH	Remarks
	October 30					
Dog No. 1...	10:00 A.M.	28.7	33.3	43.9	7.44	Violent tetany
	10:35 A.M.	56.5	22.2	27.6	7.25	CO_2 given for 20 minutes. Recovery
	3:40 P.M.	27.9	35.	42.7	7.45	Tetany
	4:05 P.M.	88.9	29.2	32.7	7.29	CO_2 given for 20 minutes. Recovery
	October 31					
Dog No. 2...	10:00 A.M.	24.1	37.6	44.7	7.46	Violent tetany
	10:25 A.M.	108.5	28.8	32.4	7.28	CO_2 administered 20 minutes
	3:50 P.M.	23.2	.....	.....	.....	Tetany
	4:15 P.M.	60.	.....	.....	.....	Dog unconscious after 20 min. Recovery
	5:00 P.M.	81.7	27.7	29.	7.16	CO_2 given 40 min.

In Table II are shown the lactic acid, CO_2 content, CO_2 capacity, and pH changes in the blood of tetany dogs before and following CO_2 treatment. We employed Riegel's (1927) modification of the Clausen (1922) sulfuric acid method for determining lactic acid.

From the data presented in Table II it seems clear that the changes in CO_2 capacity, CO_2 content, and pH of the blood are not due to CO_2 per se but to the increase in lactic acid induced by anoxemia resulting from the CO_2 treatment. The rise in lactic acid following treatment is striking and consistent, and in our opinion is sufficient to account for the observed changes in pH. The decreased CO_2 content and capacity are obviously due to hyperventilation

caused by the CO_2 treatment, with consequent blowing off of carbon dioxide.

It will be noted that four lactic acid determinations were made on dog No. 1, and five on dog No. 2. The lactic acid content of the blood is high during the recovery period and the dog remains free from tetany symptoms. When, however, the tetany again becomes manifest lactic acid was found to be at a normal concentration, i.e., about 28 mgm. per cent. The drop in lactic acid might have occurred some time before the first tetany symptoms became manifest.

Macleod (1921) observed that within an hour after asphyxia and anoxemia were terminated the blood lactic acid returned to the normal level. Table II shows that tetany reappeared five hours after the two animals were taken from the CO_2 chamber and allowed to breathe outside air. However, some of our cases developed tetany within an hour following treatment. It is very likely that in such cases the lactic acid content of the blood had returned to the normal level very quickly after removal from the CO_2 chamber.

Our data indicate that changing the reaction of the blood to the acid side produces an effect upon tetany which persists for some hours following a return to normal of the acid-base equilibrium. The work of Reigel (1927) on the rate of removal of lactic acid following injections of sodium lactate tends to bring out this point. Two hours after injecting an equivalent of 2,125 mgm. lactic acid, the lactic acid of the blood returned to a normal concentration while the inorganic phosphate of the blood remained at a much reduced figure for some time following.

The question arises, how does a shift in the reaction of the blood toward the acid side bring about a cessation of tetany symptoms? It appears probable that such a shift in the acid base balance produces profound changes in certain constituents of the body fluids. Thus Freudenberg and György (1921) have shown that calcium carbonate in solution is able to supply the correct amount of ionic calcium of the blood, the ionic calcium being one fourth the amount of total calcium. Furthermore they have demonstrated that the amount of ionic calcium present in the blood is dependent on the hydrogen ion and bicarbonate concentration. With a decrease in pH there occurs an increase in ionic calcium. Following parathy-

roidectomy it is probable, as Greenwald (1926) suggested, that the fall in total and ionized calcium is the result of a precipitation in the tissue of tertiary calcium phosphate which Holt, La Mer, and Chown (1925) have shown to be present in excess in the blood of normal animals. Stewart and Percival (1927) working on eviscerated cats found a marked rise in total calcium of serum after injection of parathyroid hormone showing clearly that the calcium must come from the tissues and not as a result of an increase in calcium absorption in the intestine.

The increase in lactic acid content of the blood brought about by anoxemia in presence of an excess of CO_2 is sufficient to account for the low pH and low CO_2 content of the blood. This increased acidity probably increases the ionic calcium concentration and increases the excretion of phosphates. An increased excretion of phosphates after the administration of hydrochloric acid and hydrochloric acid-producing substances to children with tetany has been reported by Freudenberg and György (1921) and Gamble and Ross (1925). Anderson and Graham (1924) found that the inorganic phosphate of the blood is decreased following the ingestion of ammonium chloride.

In the experiments reported here, it is probable that the acidosis caused by asphyxia and anoxemia has a greater effect on the ionized calcium than upon the total calcium. The slight increase in total calcium observed in some cases is probably due to the increased solubility of calcium phosphate at the low pH. The relief of experimental tetany by acidosis is a simple and effective method of treatment, and produces its effect by rendering the available calcium of the blood more diffusible and probably stimulates excretion of excess phosphates.

PROTOCOLS

Dog No. 38. Rat terrier, female, weight 8.5 kilos. Thyroparathyroidectomized March 22, 1927, 4:00 P.M. Tetany appeared 9:00 P.M., March 25. Animal very spastic, marked tremors and jerking of flank and leg muscles. Panting, salivation. Bled from heart. 15 c.c. taken. Serum Ca 6.5 mgm. per 100 c.c., CO_2 content 48, CO_2 capacity 56, pH 7.36. Animal placed in CO_2 chamber 9:30 P.M. and left there 25 minutes. Removed from chamber unconscious. Bled. CO_2 content 31.1 vols. per cent, CO_2 capacity 31.4 vols. per cent, pH 7.14. Serum Ca 7.6 mgm. Animal quickly regained consciousness and exhibited no signs of tetany. Ran about the laboratory, ate meat, and engaged in a fight

with another dog. At 11:40 P.M. dog bled again. Serum Ca 7.6. mgm. This animal remained free from any signs of tetany for fifteen hours after removal from CO_2 chamber. This dog died of tetany March 28 at 9:00 A.M.

Dog No. 39. Fox terrier, male, weight 7 kilos. Thyroparathyroidectomized March 20 at 2:00 P.M. Remained normal until March 23 at 8:00 A.M. Exhibited violent tetany. Serum Ca 6.7 mgm., CO_2 capacity 47.3 vols. per cent, CO_2 content 40.6 vols., per cent, pH 7.35. Placed in CO_2 chamber for 30 minutes. Taken out unconscious. Bled. Serum Ca 7.2 mgm., CO_2 capacity 28 vols. per cent, CO_2 content 25.1 vols. per cent, pH 7.23. Animal recovered consciousness within two minutes after removal from chamber. No visible signs of tetany. Animal behaved in normal fashion and remained free from tetany for 7 hours. When violent tetany symptoms again developed the animal was again placed in the CO_2 chamber and after removal all tetany symptoms had disappeared. This animal died of tetany thirty-six hours later.

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Effect of Acidosis in Strychnine Poisoning.

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It has been demonstrated by a number of investigators that changes in the reaction of the blood toward the acid side prevent the clonic contraction and spasticity of the skeletal muscles in parathyroid tetany. The experiments reported here show that a decrease in the pH of the blood produced prior to the administration of lethal doses of strychnine, prevents violent tetanus, convulsions and death.

Acidosis was produced in 7 dogs by subcutaneous injections of nephro-toxic substances such as uranium nitrate and sodium tartrate. When the pH of the blood dropped to about 7.2 and the CO_2 capacity to around 22 volumes %, the dogs received subcutaneously 1/160 gr. per kilo of strychnine sulphate. Ten minutes later slight muscle tremors and spasms developed. In all cases recovery occurred within 45 minutes. One dog died 1 hour following injection of the lethal dose of strychnine. In this case death was apparently due to uranium nitrate poisoning.

In other series of dogs acidosis was produced by means other than those involving impairment of renal function. This was brought about by administering NH_4Cl to one series of animals and CO_2 to another. Muscle spasms from which the dogs soon recovered, followed the injections of a lethal dose of strychnine.

Control dogs receiving the same lethal dose of strychnine died in convulsions within 1 hour.

From the results we obtained it is evident that it is the initial drop in pH occurring before injection of the strychnine and not the fall produced by convulsions and the resulting anoxemia that is effective in preventing convulsions and death from strychnine pois-

oning. The data from 1 of our experimental and 1 of our control dogs are presented in Table I.

TABLE I.

Animal	Date	Time	Strychnine given in gr.	pH	CO_2 capacity vol. %	Remarks
Dog 12						Given 100 cc. 5% so-
14 kilo	Feb. 28	10:30 A.M.		7.39	46.8	lution NH_4Cl by stomach tube.
		1:30 P.M.		7.19	21.1	
		2:45 P.M.	7/80			
		2:55—				
		3:20 P.M.				Spasms and muscle twitching.
		3:23 P.M.		7.04	17.5	
		3:35 P.M.				Recovered. Remained normal.
Dog 3C						
8.75 kilo	Feb. 27	3:00 P.M.		7.38	43.2	
		4:10 P.M.	1/20			
		4:20 P.M.				Violent convulsions.
		4:40 P.M.		6.92	28.3	Violent convulsions.
		5:20 P.M.				Died in convulsions.

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4039

Effect of High Temperature on the Gonads of Frog Larvae.

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Frog larvae *(R. sylvatica)* which had been raised under nearly optimal conditions up to the 7th week were subjected after the 34th day to the maximal temperature they can endure permanently, *i. e.*, 32° C. The gonads of both males and females grow much slower than those of normal controls. However, the spermatogonia of the testis are of about normal size and the seminiferous tubules differentiate in a typical way. The reactions of the ovaries are more complex and more important. At the time when the temperature was raised the ovaries contained large numbers of young ovocytes in the synapsis stage. Two weeks later this type of germ cells has completely disappeared. The ovogonia are still present and the deeper layers of the cortex contain now large auxocytes. The latter, however, are in way of degeneration. Their nucleoli are extremely large and basophilic and the ovoplasm contains coarse granules. During 2 more weeks the eggs disappear completely, the ovogonia being the only germ cells spared from degeneration.

Immediately after the differentiation of further ovocytes has come to an end, the medullary part of the ovaries starts to grow rapidly. Within a few weeks the ovarial sac *(rete ovarii)* transform into a typical rete testis, vasa efferentia and seminiferous tubules. At first the latter lack completely any germ cells. However, sooner or later the ovogonia migrate from the cortex to the medulla, and after having entered the seminiferous tubules transform into spermatogonia.

Thus the experiment leads to the total reversal of sex of the females. The normal controls consisted of 100 females and 96 males. The experimental group contained no typical females after

the 2nd week of rise in temperature; but between the 15th and the 33rd day 53 females in different stages of sex transformation and 62 typical males were preserved.

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4040

Interrelation of Pituitary and Thyroid in Metamorphosis of Neotenic Amphibians.

W. R. INGRAM. (Introduced by W. W. Swingle.)

From the Zoological Laboratory, State University of Iowa.

While it has been known for some time that transplants of anterior lobe of the pituitary of adult frogs would cause accelerated growth in small tadpoles[1] no attempt has been made to show the relation of this gland to the condition of partial neoteny existing in some anurans. Neotenic amphibians exhibit prolonged retention of larval form. Total neoteny, of which the Mexican axolotl is an example, is characterized by the failure of such animals to metamorphose spontaneously. Among the anurans *Rana catesbiana* and *Rana clamata* show greatly prolonged larval stages which mark them as partially neotenic types. In the totally neotenic axolotl, metamorphosis upon injection of anterior lobe extracts has been reported by some workers[2, 3] and denied by others.[4]

The anterior lobe of adult *Rana pipiens* was transplanted intraperitoneally into *Rana clamata* larvae of various stages of limb development—up to a hind-limb length of 5 mm. Three transplants were made at weekly intervals into 35 tadpoles, allotted according to size into cultures of 5. Controls of approximately the same development were injected into bits of muscle. After the 2nd transplant marked limb growth was noted; the general growth being accelerated to any extent only in the smaller tadpoles.

After the 12th day following the first transplantation marked metamorphic signs appeared, and in from 25 to 33 days forelimbs

[1] Allen, B. M., *J. Physiol. Zool.*, 1928, i, 153.

[2] Hogben, L. T., *Proc. Roy. Soc. B.*, 1923, xciv, 204.

[3] Uhlenhuth, E., *Brit. J. Exp. Biol.*, 1927, v, 1.

[4] Smith, P. E., *Brit. J. Exp. Biol.*, 1926, iii, 239.

began to break through and tail atrophy was observed. Most of the experimental animals were killed when they showed practically complete metamorphosis and the thyroids removed for examination. The control animals exhibited only a slight normal growth and development and in no case showed any signs of metamorphosis.

Pronounced development of the thyroids of the treated animals was found—in some cases their diameter was twice that of the control glands. Sections of the control glands displayed large, colloid-filled follicles with walls composed of flat, inactive-appearing cells. The thyroid of treated animals, on the other hand, showed considerable loss of colloid substance from the follicles with collapse of some of the latter, and extreme activity of the cells which were greatly elongated, with proportionate thickening of the follicle walls. Freeing of colloid and heightened activity were evidently effects of the anterior lobe transplants.

The histological picture approximates that found by Uhlenhuth[5] in the thyroids of normally metamorphosing salamanders and in the case of one axolotl treated with anterior lobe powder.

The evidence would indicate that the neotenic condition in certain anurans depends upon a defective relationship between the anterior lobe of the pituitary and the thyroid. Whether this depends upon a retarded anterior lobe development or upon a failure to release the anterior lobe active principle may be shown by subsequent experiments.

[5] Uhlenhuth, E., *Roux' Arch. Entw.*, 1927, cix, 671.

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4041

Comparison of Rates of Killing of Parthenogenetic and Sexual Forms of Daphnia magna at Higher Temperatures.

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From the Zoological Laboratory, State University of Iowa.

In a recent paper[1] the temperature characteristics for the rate of killing of 2 species of cladocerans were reported. One of these species, *Moina macrocopa*, yielded a value of the temperature characteristic equal to about 108,500 calories, for the temperature interval of 42° to 47° C. The second species, *Daphnia pulex*, yielded a slightly higher value, 119,500 calories, for the temperature interval 32° to 37° C. The animals used in these experiments were adult parthenogenetic females. The present paper presents data for the parthenogenetic females of a 3rd species, *Daphnia magna*, and an account of a comparison of this parthenogenetic form with the sexual females and the adult males of the same species.

The procedure used in determining the rate of killing in the present case was the same as that described in the paper just referred to. Curves of the velocities of killing at each of several lethal temperatures were determined separately for the 3 forms used. The parthenogenetic females were reared from young females placed in plenty of food. The males were reared as young from parthenogenetic females which previously had been somewhat crowded together in rich food; the sexual females were obtained by crowding females together in weak food. The rate of killing was taken as the reciprocal of the time necessary to kill 50% of the animals (Table I). To determine the temperature characteristic, the logarithms of these rates were plotted against the reciprocals of the absolute temperatures (Fig. 1) and the value of the constant μ calculated from the

[1] Brown, L. A., and Crozier, W. J., *J. Gen. Physiol.*, 1927, xi, 25.

slope of the line drawn through the plotted points according to the equation,

$$K_2/K_1 = e(\mu/R)\ (1/T_1 - 1/T_2)$$

where K_1 and K_2 are proportional to velocity constants at the respective temperature T_1 and T_2, R the gas constant, and μ the critical thermal increment or temperature characteristic.[2]

The graphs show that the thermal increment for the parthenogenetic and sexual females is practically the same, with μ equal to 187,700 calories. The plotted line gives a better fit for the parthenogenetic females than for the sexual females. This may be due in part to the fact that fewer animals were used in determining the velocities of killing in the latter form, but probably is due to the greater difficulty in getting animals of equivalent ages in the case of the ephippial females. The thermal increment for the males is somewhat different than that for the females, due to the presence of a "break"[3] at 37°. The exact slope of the graphs above and below this point cannot be given much weight, because the temperature range is limited. On the whole the sexual female more nearly re-

TABLE I.

Showing data for rates of killing of parthenogenetic females, sexual females and males of *Daphnia magna* at temperatures 35° to 41° C. The times given are those for 50% dead. The durations of exposure at each temperature ranged from those that killed 100% down to those that failed to kill the animals at all.

°C		Number of animals	Time in seconds	1/time x 10^4 (Rate)
35	Parthenogenetic females	376	5280	1.89
	Sexual females	238	7200	1.39
	Males	251	8160	1.23
36	Parthenogenetic females	503	2460	4.07
	Sexual females	204	4860	2.06
	Males	175	3600	2.78
37	Parthenogenetic females	430	600	16.7
	Sexual females	272	1440	6.94
	Males	560	1440	6.94
38	Parthenogenetic females	504	260	38.5
	Sexual females	275	336	29.8
	Males	386	288	34.7
39	Parthenogenetic females	409	104	96.2
	Sexual females	168	118	84.7
	Males	145	56	179.0
40	Parthenogenetic females	350	40	250.0
	Sexual females	148	65	154.0
	Males	161	33	303.0
41	Parthenogenetic females	335	23	435.0

[2] Crozier, W. J., *J. Gen. Physiol.*, 1924, vii, 123.

sembles the parthenogenetic female, according to its temperature characteristic, than it does the other sexual form, the male.

It is interesting to note that data for the parthenogenetic females of this species from earlier work of v. Transehe,[4] when plotted in the same manner, shows a break at 38° with a comparable value (177,500) of the temperature characteristic above this temperature. An exact comparison cannot be made, however, as he used a different end point for the death of his animals than was used in the present experiments.

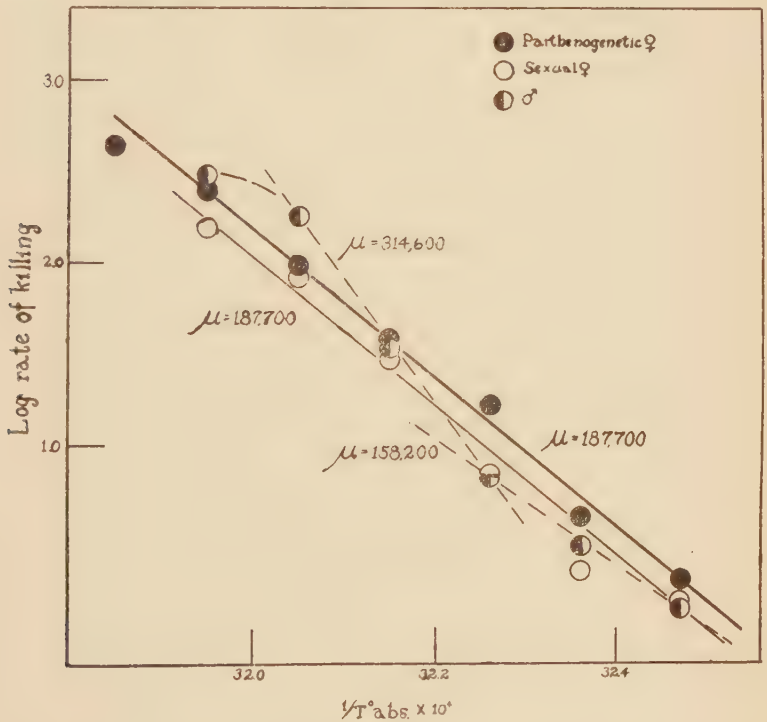

FIG. 1.

Logarithms of the rates of killing (see Table I, last column) of parthenogenetic females, sexual females, and males of *Daphnia magna* plotted against the reciprocals of the absolute temperatures. The temperatures Centigrade range from 35° to 41°. The values of the thermal increments (μ) are calculated from the equation given in the text.

[3] Crozier, W. J., *J. Gen. Physiol.*, 1925, ix, 525.

[4] von Transehe, N., *Arch. ges. Physiol.*, 1913, cliii, 323.

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4046

Effect of Various Ions upon the Heart Rhythm of Crayfish (Cambarus clarkii).

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Using perfusing solutions of various salts approximating in ionic concentrations those found by Griffith[1] to be present in the blood of *Astacus fluviatilis,* an attempt was made to determine the effect of the Na, K, Ca, and Mg ion upon the intact heart of the Crayfish *(Cambarus clarkii).* For comparative purposes a control perfusing solution containing 1% NaCl+0.028%—KCl+0.024% $CaCl_2$ was employed. Such a solution applied to the heart maintained the normal rhythm for a period of several hours when perfused at a temperature of 25±0.1 degrees centigrade. Graphic records were obtained of the heart rhythm while being perfused with the various solutions.

In determining the effect of the Na ion, 3 perfusing solutions were employed, viz.: A pure solution of 1% NaCl, one containing 2% NaCl+0.028% KCl+0.024% $CaCl_2$ and one containing 0.028% KCl+0.024% $CaCl_2$. Both solutions containing Na produced a marked increase in rate and amplitude of the beats coupled with a loss in tone. The pure 1% solution had an especially marked effect upon the tone. The solution lacking Na produced a sharp increase in the tone accompanied by a loss in amplitude and rate, the heart generally stopping in diastolic arrest in less than 20 minutes. It may be noted in this connection that Carlson,[2] working upon Limulus, found that the Na stimulates the heart and increases the amplitude of the beats. Hogben[3] noted that in Maia and Homarus a pure solution of NaCl produced an almost immediate increase in tone, resulting shortly in systolic arrest. Rogers[4] observed that

[1] Griffith, A. B., "The Physiology of the Invertebrata." New York, 1892.

[2] Carlson, A. J., *Ergebnisse der Physiol.*, 1909, viii, 372.

[3] Hogben, L. T., *Quar. J. Ex. Physiol.*, 1925, xv, 263.

[4] Rogers, C. G., *J. Exp. Zool.*, 1905, ii, 237.

NaCl had the power of restoring rhythmatic contractions in the heart of the crab *(Brachnotus nudus)*. It is concluded from the present work that the Na ion increases the rate and amplitude and decreases the tone of the crayfish heart and is, therefore, concerned in the regulation of normal heart rhythm.

Similar experiments were performed with solutions of KCl and $CaCl_2$. A pure solution of 0.024% $CaCl_2$ produced a sharp increase in tone and a slight decrease in the rate. Increasing the $CaCl_2$ the control perfusing solution affected the rate only, the latter being decreased slightly. A pure solution of 0.028% KCl invariably depressed the rate and amplitude but had slight if any effect upon the tone. The removal of K from the control perfusing solution resulted in systolic arrest of the heart in less than 30 minutes. Normal rhythm could subsequently be restored only upon the replacement of KCl in the perfusing solution. In regard to the effect of K and Ca upon the heart rhythm of other closely related species, Hogben[3] finds that in Maia an excess of K produces systolic arrest of the heart. Carlson[2] observed that K and Ca both produced depressor action upon Limulus heart muscle. The present work is similar to the above in that K and Ca depress the rate and amplitude and increase the tone of the Crayfish heart, the Ca ion having an especially pronounced effect upon the tone.

The intact heart of the Crayfish was found to beat normally in the absence of Mg from the perfusing solution. Small additions of $MgCl_2$ to the control perfusing solution had no effect upon the normal rhythm. However, when the concentration of $MgCl_2$ was considerably increased (to about 0.1%) the rate decreased somewhat. The results obtained indicate that the Mg ion is not essential for the maintenance of normal heart rhythm in the Crayfish.

Normal perfusing solutions in which the hydrogen ion concentration had been altered by means of HCl, CO_2, and NaOH produced no marked effects upon the normal heart rhythm within the range of pH 5.5 to 9.0. Decreasing the pH below 5.5, however, resulted in a marked increase in tone. Hogben[3] likewise noted that the hearts of *Homerus* and *Maia* were extremely insensitive to changes in hydrogen concentration and that the heart would continue to function normally in a perfusing solution having a pH as low at 5.6. It is evident, therefore, from the present results, that the crayfish heart is capable of marked adaptation in this respect and can maintain its normal rhythm within a considerable range of hydrogen ion concentration.

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THE ACID INTOXICATION OF ADRENAL INSUFFICIENCY IN DOGS

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Considerable work has been done in recent years on the functional significance of the suprarenals, but not until comparatively recently have any results been forthcoming which would justify the expenditure of time and energy involved. Numerous investigators turned their attention to the cortical portion of the suprarenal following the work of Biedl (1903) which clearly demonstrated the incompatibility of removal of all cortical tissue with life. This work has been amply confirmed by later investigators (Wheeler and Vincent, 1917; Houssay and Lewis, 1923; Wislocki and Crowe, 1924; Zwemer, 1927; Swingle and Eisenmann, 1927), all of whom demonstrated that complete removal of adrenal cortical tissue results in early death.

The investigation of Swingle and Eisenmann was carried out on cats, but owing to the fact that cats are very difficult to bleed and, furthermore, that large quantities of blood are necessary for repeated analysis, their results, as the senior author states, are not as complete as might be desired. A review of their tables and protocols reveals that an uncompensated, non-volatile acidosis exists in adrenalectomized cats. It was with the idea of studying the nature of this acidosis following suprarenalectomy, and especially the beginning and intermediate stages, that this investigation was undertaken.

Because of the large quantities of blood necessary for repeated analyses and because of the relative ease with which a larger animal can be bled, the dog was chosen as a subject for this investigation.

The writer takes this opportunity to express his gratitude to Dr. W. W. Swingle not only for suggestion of the problem but also for his encouragement and whole-hearted enthusiasm during the progress of the work.

Material and methods. Only adult and non-pregnant dogs were employed since pregnancy is known to greatly prolong the survival period of some dogs following ablation of cortical tissue (Stewart and Rogoff, 1927). Whereas the normal survival period ranges from $5\frac{1}{2}$ to 6 days, pregnant dogs are on record with survivals into the 57th and 58th days.

TABLE 1

Blood findings following bilateral suprarenalectomy in dogs

CASE	DATE	TIME	CO_2 CAPACITY	pH	BLOOD SUGAR	INORGANIC PHOSPHORUS	REMARKS
			vol. per cent		*mgm.*	*mgm. per 100 cc.*	
D19	January 25	10:00 p.m.					Right gland removed
Mongrel	February 4	10:00 p.m.					Left gland removed
Male	February 5	10:00 p.m.	49	7.34	105	5	Normal blood, good recovery
	February 9	11:30 p.m.			85		Vomiting, moody, not weak
	February 10	9:30 a.m.	35	7.35	62	10	Very weak, hind legs spreading. Respiration deep and full
	February 10	3:00 p.m.	23	7.17	62	10	Prostrate. Respiration 32 per minute and very deep. Died at 3:30 p.m. Survived 137 hours
D21	February 4						Right gland removed
Female	February 11	8:30 p.m.					Left gland removed
Terrier	February 13	10:00 a.m.	44.4	7.36	95	4.25	Normal, eating well
	February 15	10:00 a.m.	41.4	7.37	77		Venous.* Refused food, moody, vomiting
	February 15	2:00 p.m.	33	7.32			Respiration deeper, 24 per minute
	February 15	9:30 p.m.	28.6	7.27	83	6.75	Respiration deep with sighs
	February 16	5:00 a.m.	24.4	7.19	62	13	Prostrate. Died at 8 a.m. Survived 108 hours
D23	February 12						Right gland removed
Female	February 17	2:00 p.m.					Left gland removed
Collie	February 19	10:00 p.m.	46	7.43	109	5.3	Normal, taking meat and milk
	February 22	10:00 a.m.	38	7.42	67		Refused food, moody and weak
	February 22	10:00 p.m.			78		Respiration 20 per minute, vomiting
	February 22	12:00 p.m.	37	7.40			Weaker. Respiration 24 per minute. Deeper
	February 23	3:30 a.m.	29	7.17	64	10	Restless, "flat." Died 4 a.m. Survived 134 hours

D24	February 19						Right suprarenal removed
Brown	February 25	11:30 a.m.					Left suprarenal removed
Bull	February 27	2:00 p.m.	40.5	7.38	100	4.5	Normal, eating salmon, drinking milk
	February 28	3:00 p.m.	32.3	7.36	76 9		Quiet, moody, walks little, no food, respiration shallow
	February 28	11:45 p.m.	31.4	7.30	86.9		Tired and cuddled up. Respiration deep and full, 20 per minute
	March 1	10:30 a.m.			62.5		Breathing deeper, 24 per minute. Milk forced
	March 1	2:30 p.m.	27.6	7.27	80		Respiration 40 per minute. Lying flat at 3 p.m.
	March 1	4:00 p.m.	19	7.24	55	6.3	Involuntary bowel movement. Died at 4:30 p.m. Survived 101 hours
D26	March 2						Right suprarenal removed
Female	March 12						Left suprarenal removed
Spitz	March 14	9:30 p.m.	40.3	7.38	111.1	4.72	Normal, eating and drinking
	March 15	9:30 p.m.			100		Muscle twitchings
	March 16	4:00 a.m.	37.4	7.32	80		No food, hind legs weak
	March 16	9:00 a.m.			93		Cerebellar symptoms swaying
	March 16	2:15 p.m.	37	7.34	94		Eating meat again. Twitching of hind limb muscles
	March 17	10:00 a.m.	35	7.33	108		Eating, yelling fits. Respiration shallow
	March 18	9:30 a.m.	27	7.27	76.8		No food, legs very weak. Respiration deep, 14 per minute
	March 18	7:15 p.m.	22	7.10	66	11.12	Venous.* Respiration deep and full. Survived 148 hours
D27	March 18						Right gland removed
Male	March 26	3:00 p.m.					Left gland removed
Spitz	March 28	8:00 p.m.	46	7.36	105	4.3	Eating meat, running around
	March 30	9:00 p.m.	39	7.31	83		No appetite, tired and moody
	March 31	9:15 a.m.	29	7.28	87	5.6	Muscle twitchings, respiration 16 per minute, deep and full, 50 cc. blood drawn
	March 31	11:00 a.m.	27	7.10	64	7.7	Respiration very deep and full, 22 per minute. Death hastened by excessive bleeding at 9 and 11 a.m. Survived 116 hours

* Venous blood is two (2) volumes per cent higher than arterial blood.

TABLE 1—*Concluded*

CASE	DATE	TIME	CO_2 CAPACITY	pH	BLOOD SUGAR	INORGANIC PHOSPHORUS	REMARKS
			vol. per cent		*mgm.*	*mgm. per 100 cc.*	
D28	January 2						Left suprarenal removed
Male	January 10	Noon					Right suprarenal removed
Mongrel	January 13	8:00 p.m.			117	3.8	Fine shape; blood for CO_2 and pH lost in centrifuge
	January 15	2:00 p.m.	51	7.39			Very good condition, very active
	January 18	7:30 p.m.	34	7.35	76		At 4 p.m. weak and staggering. No food-glucose injected
	January 19	12:45 a.m.	29	7.19	91	12.5	Respiration very deep and full. Distressed and "flat"
	January 19	1:30 a.m.					Too much blood loss. Died in convulsions. Survived 205 hours
D30	March 2						Left gland removed
Male	March 9	9:00 a.m.					Right gland removed
Mongrel	March 13	8:00 p.m.					Eyes bloodshot but no weakness or food refusal
	March 14	3:00 p.m.	33.6	7.30	87	9.7	Weak and moody but took little milk after bleeding. Fed by stomach tube. Walks well but not far. Inclined to lie around
	March 15–16						
	March 17	9:00 a.m.	18.4	7.22	60	16.2	Prostrate and approaching coma. Died after bleeding. Survival—192 hours
D37	April 9						Right suprarenal removed
Male	April 14	3:00 p.m.					Left suprarenal removed
Mongrel	April 16	10:00 a.m.	45	7.41	91	3.1	Normally acting dog
	April 18	2:30 p.m.	34	7.42	78	5	No food, respiration deep. Dog moody and lying about
	April 18	8:00 p.m.			77	6.25	Vomiting. Respiration 16 per minute
	April 19	10:30 a.m.	32		77		Very deep respiration. Very moody
	April 20	4:00 p.m.	28	7.16	62	7.5	Prostrate. Died at 5:30 p.m. Survived 146.5 hours

In this work one pregnant dog was encountered which lived, however, only 9 days. At autopsy this dog showed 4 very young embryos. Another bitch after 27 days' survival was sacrificed and very careful autopsy revealed only one suspicious looking nodule which upon sectioning, however, had no semblance of adrenal tissue. Through accident the dog was removed from the animal room before early pregnancy could be ascertained so this animal remains unclassified. It is obvious that pregnant dogs could not have been employed in this series of experiments without seriously complicating the interpretation of results.

The removal of the second adrenal followed the first operation after a lapse of 5 to 10 days, or, as in some cases, after a longer interval. Longer intervals between operations apparently do not prolong the survival period of dogs. Ordinarily no great difficulty was experienced with gland extirpation, although occasional animals presented some difficulty in removal of the right gland. The same method of approach and removal was employed as reported by Banting and Gairns (1926).

All blood was drawn directly from the heart without allowing the animal to struggle. Blood drawn during struggling was discarded since muscular activity is known to alter the level of certain blood constituents including lactic acid, CO_2 and blood sugar. Blood for CO_2 capacity and pH was drawn under oil and determination of these factors was made before inorganic phosphorus and blood sugar were estimated. Normal blood was at first drawn from unilaterally operated animals since this blood presents no change from normal (Swingle, 1927; Lucas, 1926). Later, however, normal blood was obtained after the second operation. Doubly operated dogs of average survival are always normal for several days following removal of the second gland. They eat and occasionally fight with one another just as do unoperated or unilaterally operated animals. The blood pictures of these animals show no deviations from the normal blood picture of unoperated or unilaterally operated dogs (Yonkman, 1927), supporting the contention of other investigators (Rogoff and Stewart) that doubly operated dogs show complete operative recovery in that they are normally acting animals. Following the onset of characteristic symptoms of adrenal insufficiency as described below and elsewhere (Rogoff and Stewart, 1926; Zwemer, 1927), blood was taken at various intervals such as time of mild symptoms, more severe symptoms and terminal stages of adrenal insufficiency.

The CO_2 capacity of the plasma was determined by Van Slyke's (1917) method and the pH by means of Myer's bicolorimeter (1922) using Muntwyler's (1927) correction of 0.30 for dog plasma. Inorganic phosphorus of the serum was determined by the Benedict-Theiss (1924) method and blood sugar according to the method of Folin and Wu (1919).

Symptoms. The symptoms of suprarenal insufficiency are readily

observed in the dog, the first to appear being those of anorexia and weakness. Weakness is evident from the slight swaying that may be seen as the dog walks about. Often the animal walks but little and soon drops into a huddle in its cage. The animal is not as active as when no symptoms are present and often the weakness may be accompanied by refusal of food. Occasionally at this stage the dog attempts to chew or suck at a rarebit but the jaws are too weak to complete the process.

Following the onset of symptoms the dog becomes restless, carries a worried expression, is hypersensitive and often objects to being disturbed. When made to stand, the legs tend to spread and the head droops. The eyes become bloodshot, and pupils dilate and the animal presents a condition that can be no better described than by the expression—"hang-dog." Very often the animal upon being disturbed runs wobbling and swaying over to a corner of the room or back of a cage and there presents the typical "hang-dog" condition or slouches down in apparent distress. Heavy sighs give evidence that the effort required much exertion—the respiration often becoming faster and irregular.

Yelling fits of a peculiar type often accompany the condition of hypersensitivity. The least disturbance sends the dog on a wild excursion, bumping into cages and walls and from all appearances and from the noises elicited one would conclude that the animal was suffering from some wild hallucination. Rogoff and Stewart (1926) describe the same peculiar yelling fits and suggest the possibility of intestinal worms being at the seat of the disturbance. They further state that they believe some of the yelling fits are actually due to adrenal deficiency. Some dogs in this laboratory have been observed to suffer from yelling fits but at autopsy showed no worms in the entire intestinal tract. This condition was especially true of pups which in some cases suffered from these fits even after unilateral operation. These wild yelling fits are never observed in cats and were observed only in some of the dogs included here. Thus it would seem that some factor, other than the disturbance of the gut tract, may be the cause of this nervous phenomenon. What this factor may be is only a matter of conjecture unless it be suprarenal insufficiency.

The refusal of food may often be accompanied by vomiting of varying degree. In some cases vomiting may be delayed until marked weakness prevails. The vomitus is usually yellow and viscid and is often frothy and may contain blood. Not infrequently at time of vomiting bloody stools may be passed, the amount of blood increasing with severity of symptoms.

Forcing of fluid may be beneficial in some cases after food has been refused provided the forcing is not too long delayed. Once vomiting occurs early death is probable and any attempts at temporary relief by feeding milk and glucose or other liquid nutriment are usually futile.

Early symptoms may appear 24 to 36 hours before death but at times the decline is very abrupt. D22 is an animal which first showed symptoms at 10 p.m. of the 4th day of the survival period. It was judged from the blood picture that the dog would survive the night but at 8 a.m. the following morning the animal was found dead in its cage. The body was still warm and death was placed at 7 a.m. This is the shortest survival period following initial symptoms that has been observed by the writer and shows how rapidly an animal suffering from suprarenal insufficiency can pass through the stage of deficiency.

After vomiting occurs an exacerbation of symptoms follows rather quickly, including noticeable changes in depth and rate of respirations. Muscular twitchings, especially in the hind legs, are often observed, the severity of the tremors increasing with the severity of the other symptoms. Prostration is of short duration, the animal being completely exhausted and after only a few hours passing into coma and dying in convulsions which are usually accompanied by involuntary bowel movement with passage of bloody contents. Little urine is passed during the period of insufficiency but when passed it is very concentrated and has a characteristic, strong odor.

Some of the animals surviving but 2 and 3 days unquestionably died from the effects of surgical shock—at least shock was a contributing factor. The symptoms of these animals are somewhat different from those of dogs surviving bilateral removal of the suprarenals for several days. The shocked dog never eats following operation; it lies around in its cage and moves but little. Its eyes seldom become bloodshot to the extent that occurs following long survival, and muscular tremors, if present at all, are much less marked. Bloody stools may be present but the typical yellowish and frothy fluid of insufficiency of long survival is lacking.

At autopsy, besides the presence of the yellow fluid described above, there are other differences which distinguish between the shocked dog and the animal suffering from typical adrenal insufficiency. The spleen and pancreas show congestion but the spleen is noticeably hard and enlarged or elongated in the long survival. The liver of the latter animal is extremely soft and mushy and the gall bladder is more distended in the long survival than in the shocked animal.

The kidneys present no evident gross change following bilateral adrenalectomy.

Blood findings. *Acidosis.* The blood findings indicate that with the onset of symptoms of adrenal insufficiency such as refusal of food, muscular weakness and general lethargy, the initial fall in CO_2 capacity occurs, whereas the pH remains at normal level or at least within the normal range. With continued fall in CO_2 capacity the pH remains normal until the capacity has fallen to approximately from 30 to 33 volumes per cent.

At this level the first decline in pH is usually observed and when the level of CO_2 capacity falls below 30 vol. per cent a rapid decline in pH ensues. The gradual decrease in CO_2 capacity is accompanied by a corresponding increase in depth, and in the later stages, in the number of respirations. There comes a time, however, when respiratory compensation is inadequate and a sharp decline in pH follows, this change occurring between 4 and 12 hours preceding death.

Blood sugar. With the onset of symptoms the initial fall in CO_2 capacity is accompanied by a marked drop in blood sugar which falls to approximately 75 to 80 mgm. per cent of blood. The possibility remains that the sugar may drop before the capacity thus not necessarily accompanying the CO_2 capacity decline. The sugar level may fluctuate materially once it begins to fall and in two of the several cases approached the normal level (D24 and D26).

D24 is a dog which showed fluctuation of the blood sugar level before and after forceful feeding. Examination of the protocols reveals slight fluctuations in blood sugar levels but none as marked as here cited. Regardless of the fluctuations the sugar level invariably drops in the acute stages preceding coma, in some cases to as low as 55 to 60 mgm. per cent. It seems probable that the early symptoms of adrenal insufficiency such as anorexia, languor and first signs of weakness are due to the initial decline in blood sugar. Every case in the protocols reveals a low blood sugar at time of first signs of weakness and the levels of blood sugar are sufficiently low in the respective animals to account for the degree of weakness noted.

Phosphorus. The inorganic phosphorus of the serum shows a gradual rise with the onset of symptoms but the rise is most rapid in the later stages. An examination of D21 shows a normal phosphorus of 4.25 mgm. per cent with a slight increase to 6.75 mgm. at the time the acidosis changed from the compensated to the uncompensated condition. Seven hours later with a pH of 7.19 the phosphorus level had risen to 13 mgm. Had the phosphorus been determined again three hours later, just one hour before death, perhaps a still higher level would have been observed. Lucas (1926) cites a dog surviving 217 hours and having a phosphorus of 26.1 mgm. This is higher than any encountered in this series of observations, the highest ever observed here being 16 mgm. per cent (D30).

Renal function tests and albumin. Excretion in the kidney was determined in three cases by means of the Rowntree and Geraghty (1910) method. In a cat living 4 days, 4½ hours, normal excretion amounted to 65 per cent. Three days after the second operation the output had decreased to 28 per cent, the animal showing slight signs of weakness. On the fourth day with marked weakness prevailing excretion had further diminished to 12 per cent.

In a dog, kidney output fell from normal 84 per cent to 60 per cent at time of first symptoms and in another cat it fell from normal 59 per cent to 37 per cent on the third day after the second operation and the cat showing no signs of weakness whatever.

The data on renal function tests are too meager to be of much significance and are included here for what they may be worth. The single case of the 4 day cat is clean cut and shows clearly a greatly diminished output of the dye following the onset of suprarenal insufficiency symptoms. However, the significance of these figures in the absence of blood pressure determinations is doubtful. The writer intends to continue this work.

In none of the bilaterally adrenalectomized dogs was any albumin found in the urine.

Discussion of blood findings. In this investigation attention was concentrated upon the CO_2 capacity, pH, inorganic phosphorus and blood sugar,—the three factors first named because they concern themselves with acidosis, and the latter because it seemed desirable to account for the initial symptoms by means of the blood picture. No determinations were made for chlorides, urea, non-protein nitrogen and other blood constituents since detailed reports concerning these components are given elsewhere (Swingle, 1927; Swingle and Eisenmann, 1927; Rogoff and Stewart, 1926; Lucas, 1926; Banting and Gairns, 1926). The rôle that some of these constituents probably play is commented upon by the respective authors.

Whatever toxic agents be present may be temporarily dispelled or diluted as shown by the work of Banting and Gairns (1926) who report transient relief following injections of saline solutions. Prolongation of the survival period of suprarenalectomized cats is effected by oral administration of various fluids (Corey, 1927) and in dogs by intravenous injections of normal saline and 5 per cent glucose (Rogoff and Stewart, 1926). Apparently the basis for all of this work is the relief of the dehydration which occurs in adrenalectomized cats and dogs and perhaps the diuretic effect of the fluids administered is a factor (Banting and Gairns, 1926). Rogoff and Stewart (1926) suggest the possibility of the intestine excreting the toxic substance or substances and further state that if this is true, the pathological condition of the gut at autopsy is largely accounted for.

As the data indicate, and as Swingle discusses in his paper (1927), there remain two possibilities accounting for the death of suprarenalectomized animals, namely, the hypoglycemia and the acidosis. That hypoglycemia is but a contributing factor and not the sole cause of death has been demonstrated by relieving the hypoglycemia with glucose injections (Swingle, 1927). Cats, thus injected, die in spite of a normal blood sugar.

Banting and Gairns (1926) removed the adrenals from depancreatized dogs and observed that their animals died with nitrogen retention and high blood sugar. These data preclude the conclusion that death results from hypoglycemia although it is evident that it is a contributing factor.

It is of interest to note that weakness and lassitude which are first to appear in the train of typical adrenal insufficiency symptoms are directly associated with a fall in blood sugar. The sugar level at this stage falls as low as 75 mgm. per cent. In most of the cases recorded there is a gradual decline in blood sugar after the initial fall occurs but D24 and D26 present evidence that the sugar level may fluctuate, apparently depending upon whether or not food is taken voluntarily or is forced.

The point worthy of emphasis, however, and bearing repetition, is that refusal of food, weakness and general fatigue seem directly attributable to a low blood sugar, this level varying with different animals. Swingle (1927) has shown that these symptoms can be partially alleviated by subcutaneous injections of glucose and insulin but the effects are transient and soon wear off, marked symptoms being associated with a gradual lowering of the blood sugar level until death supervenes.

The other possibility—that of acidosis, is of sufficient magnitude to cause death without taking into account the accompanying hypoglycemia, although the latter, as indicated, may be a contributing factor.

It was observed that acidosis could be readily followed though not with accurate nicety, by means of the visible changes in respiration. Before much decrease in CO_2 capacity had occurred no appreciable variation in respiration had taken place. However, when respiration became deeper and slower the blood picture showed significant decreases in CO_2 capacity. Approaching the level when the compensated acidosis with low CO_2 capacity and normal pH shifts to the uncompensated condition with falling pH, respiration became deeper and more rapid until in the acute stages, with lowering pH and continued fall in CO_2 capacity, respiration was deepest and most rapid until the animal passed into coma. This change in respiration and failure of respiratory compensation served conveniently as a rough indicator of the degree of acid intoxication.

That the CO_2 capacity determination of the plasma is of little value unless the pH is also considered is seen from the recent work of Pico and Deulofeux (1926) who report that following suprarenalectomy in dogs "the carbonic anhydride diminished, especially in the arterial blood because of hyperpnea." This would result in a condition of alkalosis with rising pH in contradistinction to the condition of acidosis with falling pH reported here. It is obvious that with CO_2 content and CO_2 capacity determinations the pH of the blood must be considered and that no reliance can be placed on CO_2 readings alone.

In a preliminary report (1927) it was suggested that the acidosis was

due to retention of the acid end-products of normal metabolism. Failure of the kidney to excrete acid phosphate and organic acids (Swingle, 1927) partially accounts for the acidosis following bilateral adrenalectomy in cats. In dogs the gradual rise in inorganic phosphorus of the serum plays an active rôle in the condition known as acidosis,—its major action being exerted in the uncompensated condition when the rise is most rapid. However, this increase in inorganic phosphorus is not sufficient to account for the degree of acidosis present and further light is shed on the factors contributing to the acidosis by the recent work of Swingle and Wenner (1928) who observed a marked increase in inorganic sulphate in adrenalectomized dogs. The phosphate and sulphate retention when considered together probably largely account for the degree of acidosis present. That HCl is hardly a factor to be considered as contributing to the acidosis is seen from the work of Rogoff and Stewart (1926) and Lucas (1926) who report that blood chlorides are often moderately diminished, and from Swingle (1927) who reports chlorides remain practically unchanged in cats. Future work should include determination of lactic acid and other organic acids.

The acidosis of adrenalectomy presents a marked similarity to the acidosis of chronic interstitial nephritis. In both conditions phosphate and sulphate retention contribute to the uncompensated acidosis observed in the terminal stages.

The fact that the blood picture following bilateral suprarenalectomy reveals a condition of acid intoxication, retention of nitrogenous constituents, inorganic phosphorus and inorganic sulphate, indicates that severe renal impairment of some sort probably follows as a direct consequence of loss of adrenal cortical tissue.

SUMMARY AND CONCLUSIONS

1. The blood of suprarenalectomized dogs was studied with regard to inorganic phosphorus, blood sugar, pH and alkali reserve.

2. The inorganic phosphorus of the serum rises gradually, increasing with severity of the insufficiency symptoms.

3. The blood sugar shows a decline of great significance with the first signs of weakness. The sugar level may fluctuate after the initial decline but invariably drops in the terminal stages.

4. The CO_2 capacity—a measure of the alkali reserve—gradually decreases with the onset of symptoms.

5. The pH persists at normal level until the CO_2 capacity falls to from 30 to 33 volumes per cent. At this level the first decline in pH is observed and is seen to accompany the further decrease in CO_2 capacity. This condition is indicative of the acidosis which shifts from the compensated state with low CO_2 capacity and normal pH, to the uncom-

pensated condition with low CO_2 capacity and low pH. The acidosis is in part due to phosphate and sulphate retention.

6. Renal function tests reveal diminished output by the kidney.

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STUDIES ON AMPHIBIAN METAMORPHOSIS. III. THE INFLUENCE OF THE ANNULAR TYMPANIC CARTILAGE ON THE FORMATION OF THE TYMPANIC MEMBRANE

(Five plates and four figures)

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INTRODUCTION

THE formation of the tympanic membrane and associated structures during the metamorphosis of the frog larva has been known for some time. In general, the following embryological developments and transformations occur in the formation of the middle ear of the adult frog. A posterior and dorsal process of the metapterygoid region of the quadrate is formed, and its distal end finally constricts off to form a cartilaginous ring, the annular tympanic cartilage (*ATC*, Fig. A). The annular tympanic cartilage gradually shifts posteriorly and in time becomes associated with the tympanic cavity beneath connecting with the Eustachian tube, and with the lamina propria which covers its lateral surface. Near the end of metamorphosis, and at a time when the tail is completely resorbed (Fig. B), the adult structures of the middle ear are quite well formed. The annular tympanic cartilage appears slightly circular or slightly oval in shape, and is completely covered by the translucent lamina propria. The columella extending through the tympanic cavity is seen to touch the lamina propria near its center. The external appearance of the tympanic membrane at this time is represented in Figure C.

Histologically, the tympanic membrane consists of three distinct layers. The lamina propria must be considered as the foundation layer and corresponds in shape and area to the annular tympanic cartilage to which it is attached. Internally, an epithelial layer, continuous with the mucous membrane of the tympanic cavity, is closely applied to the lamina propria; while the third and

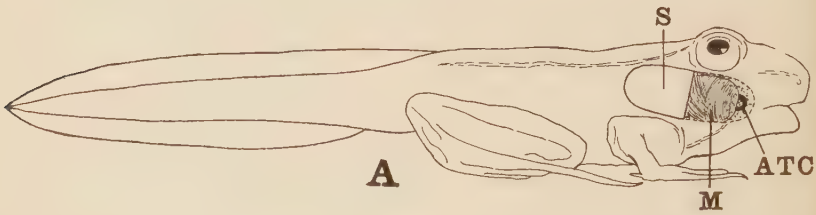
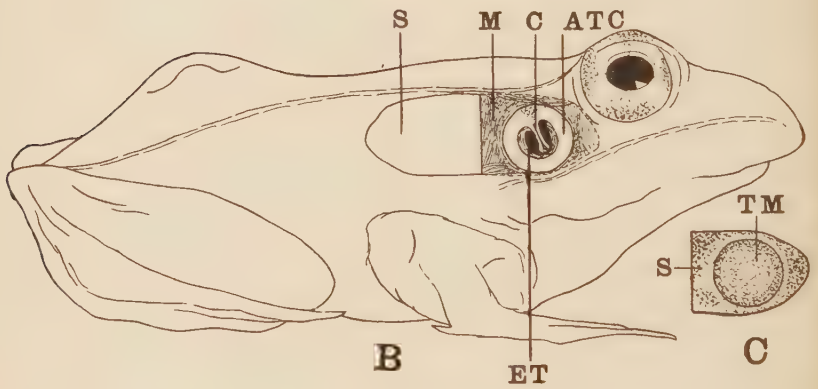


FIG. A



FIGS. B, C

external layer consists of modified integument. This latter layer is visible externally as a conspicuous, almost circular area, brownish in color and slightly indented. Its formation is gradual from normal integument and is the result of definite histological transformations.

The present paper deals, primarily, with the factors responsible for the development of this integumentary layer of the tympanic membrane[1] during metamorphosis. The fundamental question involved here is whether the thyroid hormone initiates the integu-

[1] The term "tympanic membrane," as used throughout the present paper, refers only to the *integumentary* portion of the membrane.

mentary changes responsible for tympanic membrane formation directly, through the blood stream, or whether intermediate influences are set in operation which produce the same end result. In either case the specificity of the skin for tympanic-membrane formation constitutes a second problem. That is to say, is the skin which normally develops into tympanic membrane hereditarily specific in this respect or does all integument inherit such potentialities, the skin of the tympanic membrane region undergoing the histological transformation due solely to its association with other structures of the middle ear.

As far as the writer is aware, the present paper constitutes the first attempt to explain these points regarding the development of the tympanic membrane of the anuran. Previous workers have concerned themselves, chiefly, with the histology of the adult membrane. Of these, Moldenhauer (1878) has pictured and described, quite fully, the conditions found in *Rana esculenta* and *Rana temporaria*. More recently, Tokura (1925) describes the peculiar fibrous construction of the dermal layers in the tympanic membrane of *Rana nigromaculata*, a variety of *Rana esculenta*. The correspondence of *Rana pipiens* in this respect will be taken up in a later section of this paper.

The methods of attack followed in the present work were briefly as follows: Skin transplants were made from the side and back of the tadpole to the tympanic membrane region, after first having removed the area of integument which normally forms tympanic membrane. Reciprocal grafts of skin from the tympanic membrane area transplanted to the back and side were also made on normal tadpoles. During metamorphosis, the possible formation of tympanic membranes in foreign integument or in skin of the tympanic membrane region transplanted to an abnormal location could be observed, and light thrown on the question of a possible self-differentiating system being present in the skin of the tympanic membrane region. Other series of operations involved the extirpation of annular tympanic cartilages at early and late developmental stages and their transplantation under the normal skin of the side and back. The possible influence and relationship of this structure to tympanic membrane formation was thus determined. These

operations, which will be described in detail in later sections of the present paper, included the following series of experiments:

1. Autoplastic skin transplantations (42 cases): Skin grafts from tympanic membrane region of the right side interchanged with skin grafts from the back.

2. Autoplastic skin transplantations (34 cases): Skin grafts from tympanic membrane region of the right side interchanged with skin grafts from the side.

3. Autoplastic annular tympanic cartilage transplantations (52 cases): Annular tympanic cartilage transplants beneath side skin of tadpole.

4. Autoplastic annular tympanic cartilage transplantations (65 cases): Annular tympanic cartilage transplants beneath back skin of tadpoles.

MATERIALS AND METHODS

Full-grown larvae of *Rana pipiens* were employed for the operations. They were collected during the month of July from a small pond in the vicinity of Iowa City, Iowa. Because of the ease of collecting thousands at a time, selection of the healthiest-appearing specimens was possible and helped considerably in lowering the mortality rate of those employed for operative work.

The selected specimens were placed in suitable aquaria in the laboratory and fed regularly on a diet consisting largely of *Spirogyra*. *Spirogyra*, as a preoperative food, has been found very satisfactory by the writer for all species of frog larvae. The animals fatten up within a few days, and apparently increase their vigor, as compared with tadpoles fed on other diets. This is especially useful where the tadpoles are to be metamorphosed, artificially.

In selecting tadpoles for operative work, only those of a certain size were used. For certain of the transplant operations, tadpoles in various stages of metamorphosis were selected; while in other cases, the host animals were fully grown, normal tadpoles on the verge of metamorphosis. Such individuals invariably underwent the first stages of metamorphosis within 2–5 days following the operation.

The operations were mainly carried out under anesthesia. For this a 0.05 per cent aqueous solution of chloretone (trichlortertiary-

butyl alcohol [Parke, Davis & Co.]) was employed. In general, slow anesthesia was found to be more satisfactory than rapid, although the individual resistance to the anesthetic was subject to some variation. It was found, however, that if the animals were anesthetized for about 30 seconds longer than necessary to inhibit movement, the best results would be obtained. The purpose of thus regulating the anesthetization period was to induce a period of anesthesia just sufficient to carry out the operation and allow sufficient time for the grafts to adhere.

Postoperative treatment consisted in placing the animals in shallow water so that the grafted area and wound incisions were exposed to the air for at least 15 minutes. Such exposure resulted in the congealing of the lymph and blood and the consequent adhering of the grafts sufficiently to allow total immersion of the animal. Proper anesthetization resulted in the recovery of animals soon after such immersion. The operated animals were isolated in small battery jars and fed daily with *Spirogyra*. The changing of the aquarium water each day assisted materially in reducing the mortality. Case histories for all operated animals were maintained until the individuals died or were killed for histological examination.

FORMATION OF THE NORMAL TYMPANIC MEMBRANE

1. MACROSCOPIC SEQUENCE OF EVENTS

The first external indication of tympanic membrane formation consists of the appearance of a small oval area just beneath and slightly posterior to the eye. The area appears slightly reddish in color as compared with the surrounding integument, and is of a uniform shade. Its boundaries are not marked off very distinctly, however, the gradation into normal integument being a gradual one at every point. This first indication of the future location of the tympanic membrane occurs at a rather late stage of metamorphosis. In an average 80 mm. tadpole, both fore legs have emerged, the hind legs are approximately 40 mm. in length, while the tail is about one-half atrophied. The tadpole has assumed the typical spotting of the adult frog and is seen to spend considerable time floating on the surface of the water.

As development proceeds, the small oval area tends to assume

a more circular shape, although still retaining its reddish tinge. The anterior and posterior margins, however, are now seen to be somewhat indistinctly bounded by dark grayish lines, the dorsal and ventral boundaries still exhibiting a gradual gradation into the normal integument. Further development results in the approximation of these lines dorsally, so that the area as a whole soon assumes a triangular shape and shifts further posterior. Acccompanying this configuration, the skin is seen to assume a deeper shade of red, and the area as a whole is quite distinctly marked off. The texture of the skin is still typically tadpole-like, however, and shows no signs of indentation. At this stage the tail is reduced to a mere 10 mm. stump, and the rapidly metamorphosing tadpole has assumed the sitting posture of the adult frog.

The succeeding steps now follow quite rapidly. The area soon becomes more oval in shape and, with the exception of a small, dorsal, anterior region at the periphery adjacent to the eye, is distinctly marked off from the surrounding integument. At this stage, however, two characteristics of the fully formed membrane make their appearance, namely, indentation and texture. The indentation is very slight at first and is noticeable only when the animal is viewed obliquely and under a strong light, since the depression is slight and quite gradual at the periphery. The texture of the skin is now quite different from that of normal integument. The reddish, pigmented appearance has been replaced by a finely mottled or stippled effect; and the whole membrane is distinctly light brown in color, except for a narrow gray zone at the periphery. This condition obtains when most of the external transformations of the animal are practically completed, the tail, for example, being but a small black stump about three mm. in length.

Following this, the area tends to become more circular in shape and distinctly outlined at all points. The color deepens somewhat; and by the time the tail is completely resorbed, it resembles in shade the membrane of the full-grown frog. The area is now also quite strongly indented and has shifted posteriorly and dorsally to approximately its final orientation. The external changes from this period on are slight and consist chiefly in a uniform enlargement in diameter and a gradual change in color from brown to grayish

brown. The size of the tympanic membrane increases gradually and in proportion to the growth of the adult frog.

2. HISTOLOGICAL SEQUENCE OF EVENTS

Sections made through the skin of the tympanic-membrane region at succeeding stages of metamorphosis show quite clearly that the various integumentary layers undergo an orderly process of histolysis and transformation which results in the formation of the typical tympanic membrane of the adult frog. The process is a gradual one and, once initiated, appears to progress at a uniform rate. For convenience of description, however, it may be divided into three fairly distinct transformations as pictured in Figures 1, 2, 3, and 4, Plate I.

The normal integument, as shown in Figure 1, is maintained up to a time slightly preceding the appearance of the first macroscopic sign of tympanic membrane formation. The integument is normal in all respects as compared with other side skin—skin glands pigment cells, and the various layers being fully represented.

The first signs of tympanic membrane formation are concerned with the structure of the stratum compactum layer. The layer loses its convoluted appearance, and the individual fibrils composing it tend to straighten out, so that they are more or less parallel to the surface. Considerable disarrangement of the fibril bundles next takes place, so that the layer as a whole presents a very disorganized picture (*SC*, Fig. 2). The long spindle-shaped nuclei of the normal stratum compactum appear less frequently and are either lost at this time or, more probably, covered over by the dissociating fibrils. Paralleling this dissociation, lymphocytes make their appearance and apparently migrate from the subcutaneous connective tissue into the compactum layer. They may be found at all levels but are particularly abundant where the two layers meet and are apparently engaged in removing dissociated fibrils at this point. At this stage of transformation all other layers of the skin are intact, so that the integument appears normal when viewed externally.

The dissociation of the stratum compactum progresses rapidly (Fig. 3) and in time all signs of unification are lost. The fibrils appear to group together into fused bundles and invade the subcutaneous

PLATE I

FIGS. 1–4.—Sections through the tympanic membrane region of the integument of a tadpole, showing the successive cytological steps leading up to the formation of the tympanic membrane (*Rana pipiens*). *E*, epidermis; *SS*, stratum spongiosum; *SC*, stratum compactum; *SB*, subcutaneous connective tissue; *P*, pigment masses; *L*, lymphocytes; *BV*, blood vessels; *G*, glands; *VF*, vertical fibers. ×110.

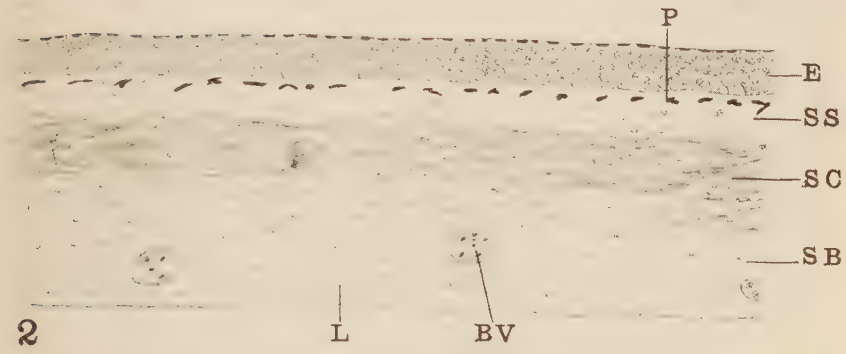
FIG. 1.—Normal integument of tympanic membrane region.

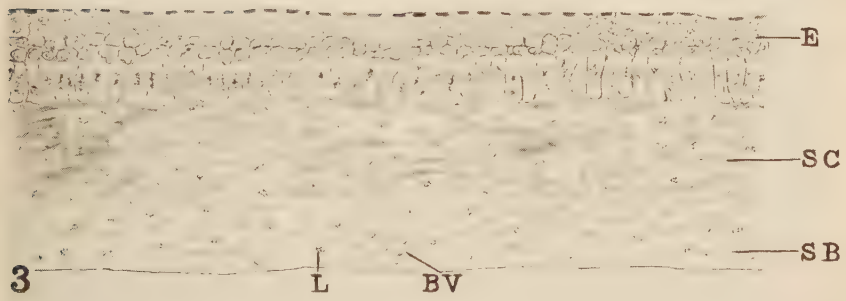
FIG. 2.—First cytological changes. Fibrils of stratum compactum lose their convoluted, wavy appearance and begin to dissociate. Lymphocytes begin to invade the area quite heavily.

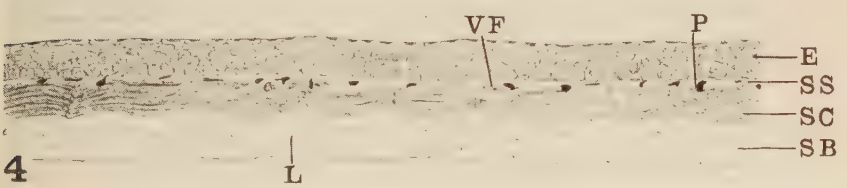
FIG. 3.—A considerably more advanced stage. The epidermis has increased in thickness, and the cells of the basal layer have hypertrophied. The stratum compactum is broken up into bundles of dissociating fibrils, which obscure the stratum spongiosum. Lymphocytes are large and active.

FIG. 4.—Appearance of the tympanic membrane at the end of metamorphosis. A narrow stratum spongiosum has appeared, while the epidermal and subcutaneous layers are reduced in thickness. The stratum compactum is composed of granulated, horizontal, and vertical fibers. Lymphocytes become scarce.

PLATE I







connective tissue. At this stage the lymphocytes are very numerous and appear quite large in size. The stratum spongiosum is entirely obliterated, and the pigment cells and glands characteristic of its normal structure are absent. In brief, all space below the epidermis, with the exception of the lower levels of the subcutaneous connective tissue, is filled with the dissociating bundles of stratum compactum fibrils. The epidermis at this time exhibits cytological changes for the first time. The cells of its basal layer hypertrophy greatly and compose nearly one half of the thickness of this layer. The cells are columnar in shape and contain large nuclei and vacuolated spaces. This condition, together with the absence of subepidermal pigment, no doubt accounts for the reddish tinge of the skin when viewed externally. The remaining cells of the epidermis appear to increase in number, so that the epidermis is one or two layers thicker at this time. The size of these cells remains quite constant, however.

Subsequent changes in the skin are concerned with the building up of structures typical of the adult tympanic membrane. The basal cells of the epidermis are either replaced by smaller cells or reduced in size (Fig. 4), so that the resulting epidermis appears normal again. The stratum compactum assumes a wavy appearance again, although it is seldom thrown into the pronounced convolutions typical of normal skin. The fibrils, however, appear to be larger in diameter and are grouped into loose bundles. Typical stratum compactum nuclei are absent, while scattered lymphocytes may still be found. Numerous small granules appear to be distributed quite uniformly throughout the layer; and it is just possible, though unlikely, that they represent the cut ends of fibrils. The resultant appearance of the stratum compactum is one not to be confused with the corresponding layer of the normal skin. Aside from the fact that it is little more than one-half the thickness of the latter, it always has a deeper staining reaction and is more closely knit together. The exact nature of its structure is interesting to consider at this time. According to Tokura (1925), it is composed of two layers of fibrous elements, viz.: an inner thick region of horizontal fibers and an outer region of smaller fibers running vertically, which cross with those of the inner region to form a complex network. This, in general, the

writer found to be true in *Rana pipiens*. Several of the outer vertical fibers are represented in cross-section in Figure 4, *VF*. At this time a thin stratum spongiosum layer makes its appearance, although it is apparently absent at certain points where the stratum compactum is in close contact with the epidermis. Where it is present, pigment cells soon develop; and occasional glands, always small in size, occur distributed unequally at different levels of the membrane. The subcutaneous connective tissue is by this time quite normal, although considerably decreased in thickness, as compared with that of normal skin. The resulting histological picture (Fig. 4), therefore, is that found at the close of metamorphosis and at which time the tympanic membrane appears as a well-defined, light-brown area, externally. In total thickness the integument is now less than one-half that of the normal integument from which it was derived.

3. UNION OF THE VARIOUS LAYERS OF THE TYMPANIC MEMBRANE WITH THOSE OF THE SURROUNDING INTEGUMENT

In the newly metamorphosed tadpole, the junction point of the tympanic membrane with the surrounding integument, although distinguishable histologically, is not very pronounced at first. As the frog develops, however, a comparatively narrow zone is evident between the two tissues which defines the periphery of the tympanic membrane histologically, and corresponds roughly in position to the rim of the annular tympanic cartilage beneath (*X*, Fig. 9, Plate III). In the fully grown frog (Fig. 5, Plate II) the following points are distinguishable in this region. The epidermis is seen to bend sharply downward, chiefly at the expense of the stratum spongiosum. This is evident, externally, as marking the boundary of the indented membrane. The epidermis from this point is also gradually reduced in thickness to conform with that of the tympanic membrane.

The stratum spongiosum of the normal integument narrows quite strikingly to join with the much narrower stratum spongiosum of the tympanic membrane. It is interesting to note here, that the skin glands, which are quite small and few in number in this layer of the tympanic membrane, may be found normal in size and abundance in the adjacent integument within a short distance of the union area. The relationship of the two compactum layers is not so clear, however. In general it may be said that most of the fibers

(horizontal fibers of Tokura [1925]) gradually merge with the fibrils of the normal stratum compactum. The latter appear to run past the rim of the annular tympanic cartilage, however, and merge with the tympanic membrane fibers a short distance in from the point of indentation of the epidermis.

That most of the tympanic membrane fibers connect with the stratum-compactum layer of the normal skin seems quite certain. If the tympanic membrane has been sectioned horizontally, however, a small portion of the outer stratum compactum of the tympanic membrane is seen to spread out, and the individual fibers appear to run into the stratum spongiosum of the normal integument (*X*, Fig. 5, Plate II). The junction area of these fibers is small in extent and occurs just beneath the indentation of the epidermal layer. These fibers generally take a deeper stain and are probably of the vertical layer as described by Tokura (1925). The subcutaneous connective tissue of the two integuments is continuous and undergoes no transformation except a slight decrease in thickness. When the cross-section has been made to include the annular tympanic cartilage (Fig. 9, Plate III), the subcutaneous connective tissue is nearly obliterated where the rim of the cartilage comes in close approximation to the stratum compactum. This is not unusual, since the subcutaneous connective tissue is subject to considerable variation in depth in different regions of the tympanic membrane.

SPECIFICITY OF THE INTEGUMENT

1. PURPOSE OF THE SKIN TRANSPLANTATIONS

The development of the tympanic membrane at a certain stage of metamorphosis, and in a location involving always approximately the same area of integument, suggested one of two alternatives regarding the specificity of integument in this regard. Either the area of integument involved is hereditarily specific in its ability to transform into tympanic membrane under metamorphic influences or else non-specific integument, by virtue of its association with structures of developing middle ear, is capable of so transforming. It is clear that if the former condition existed, then transplantation of such integument to other regions of the tadpole should not inhibit its development into tympanic membrane during metamor-

PLATE II

FIG. 5.—Section through the periphery of a newly formed tympanic membrane, showing its union with the surrounding integument. The tympanic membrane is pictured on the right side, while the normal integument is shown at the left. *X*, fibers of stratum compactum merging with stratum spongiosum of normal integument. ×110.

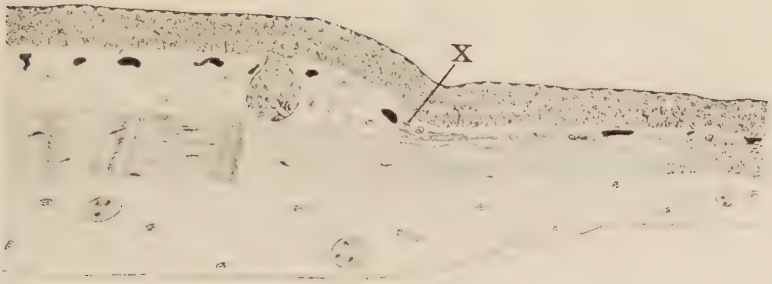
FIGS. 6–8.—Sections through developing tympanic membranes which occurred in connection with the various types of operations performed. The various layers of the integument correspond to those labeled in Plate I.

FIG. 6.—Membrane formed in back-skin graft transplanted to tympanic membrane region. The central portion is not as completely developed as the peripheral areas. (Case ABT 2.) ×110.

FIG. 7.—Membrane developing in a side-skin graft transplanted to tympanic membrane region. (Case APT 11.) ×110.

FIG. 8.—Membrane formation in side skin underneath which an annular tympanic cartilage transplant had been placed. The cytological changes are most marked in the skin directly over the rim of the cartilage transplant. *CT*, portion of annular tympanic cartilage transplant. (Case ATC 19.) ×110.

PLATE II



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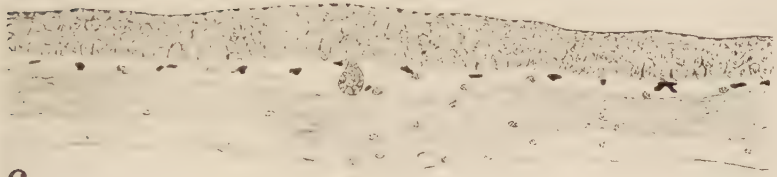

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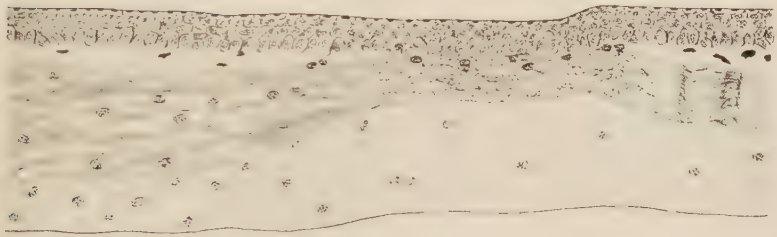

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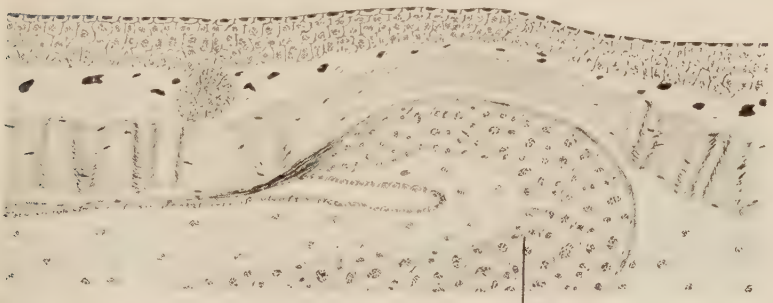

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phosis, providing, of course, the grafts so made, were well nourished in their foreign location and acquired a good blood supply. If, however, the latter alternative existed, we should expect that isolation of the integument from the developing structures of the middle ear would result in the failure of tympanic membrane formation in such grafts during metamorphosis. Reciprocal grafts would also tend to throw light on this question, in that integument of the back and side would be expected to transform normally when transplanted to the tympanic membrane region if structures of the middle ear were responsible for tympanic membrane formation. Conversely, if only the integument of the tympanic membrane region is capable of tympanic membrane formation, we should not expect foreign integument to transform likewise when transplanted to that area.

2. TECHNIQUE OF SKIN TRANSPLANTATIONS

The following technique was employed in making all skin transplantations: The anesthetized animal was placed on a damp cloth, and the area of skin to be removed was carefully cut by means of a finely sharpened pair of iridectomy scissors. Considerable care must be exercised in this regard, so as not to cut the thin peritoneum beneath. The square of skin so outlined is then grasped with a fine-pointed forceps and slowly pulled off, the connecting strands of subcutaneous connective tissue being cut with a small spear-head scalpel. Where reciprocal grafting was employed, both grafts were first freed from the peritoneum, after which they were interchanged. The only precaution necessary when adjusting the grafts is in carefully flattening out the edges, which have a tendency to curl up beneath and so retard proper adhesion.

In consequence of the tendency of skin grafts to shrink in size when removed, a marked wound area surrounding the grafts is obtained upon transplantation. In general, this necessitated the transplantation of a graft to the tympanic membrane region somewhat larger than that of the reciprocal transplant removed from that area, since adhesion and healing progress more slowly at this point than in the case of transplants placed on the back or side. It was therefore possible to fit the transplanted graft of skin quite accurately to the denuded area of the tympanic membrane region.

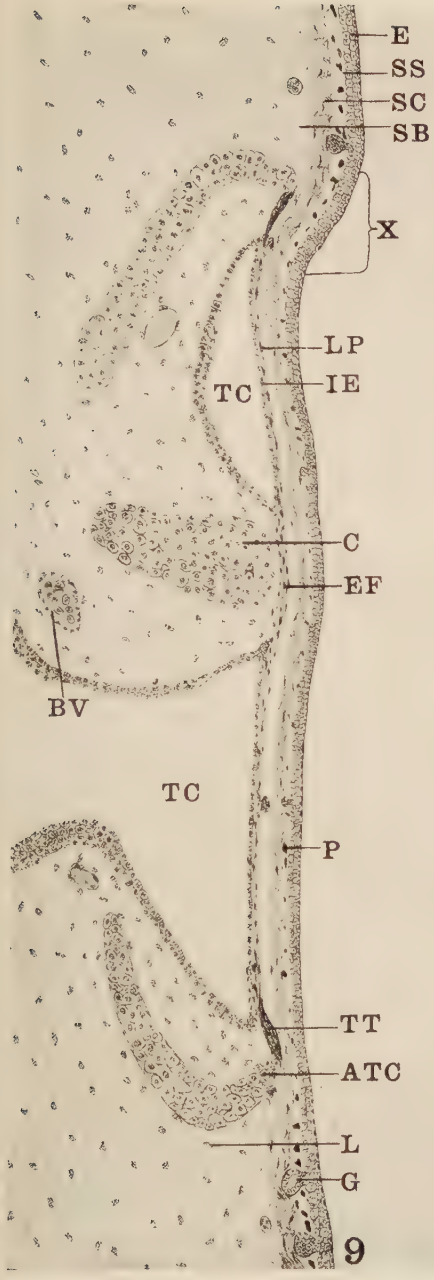
PLATE III

FIGS. 9, 10.—*E*, epidermis; *SS*, stratum spongiosum; *SC*, stratum compactum; *SB*, subcutaneous connective tissue; *LP*, lamina propria; *IE*, inner epithelial layer; *P*, pigment masses; *C*, tip of columella; *EF*, thickened elastic fiber region of lamina propria; *TT*, tensor tympani; *TC*, tympanic cavity; *ATC*, normal annular tympanic cartilage; *CT*, annular tympanic cartilage transplant; *G*, skin glands; *L*, lymphocytes; *BV*, blood vessels; *X*, transition region between tympanic membrane and surrounding integument.

FIG. 9.—Cross-section through a normal tympanic membrane, showing its associations with the columella and annular tympanic cartilage. ×30.

FIG. 10.—Cross-section through a tympanic membrane formed in the skin of the back under which an annular tympanic cartilage transplant had been grafted. The section includes the transplant, columella, and adjacent tissues. (Case CB 27.) ×40.

PLATE III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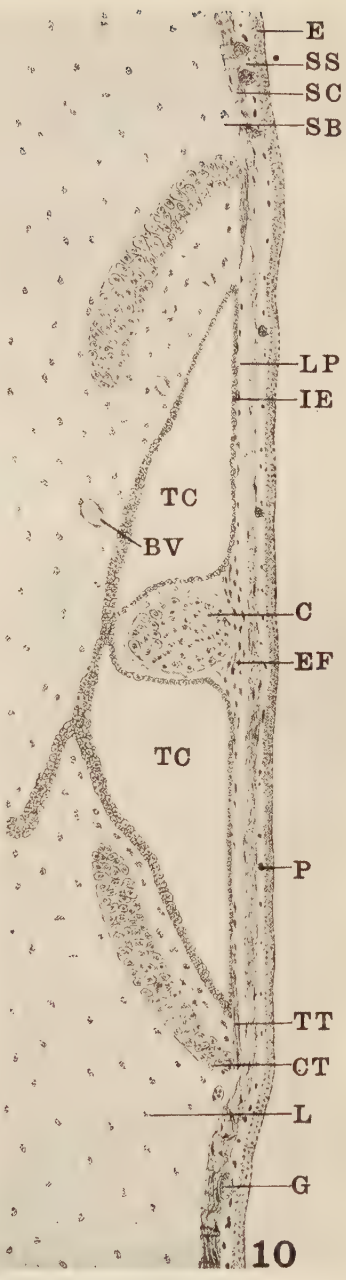
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This, obviously, resulted in relatively large wound areas surrounding the grafts placed on the back and side.

Following the adjustment of the grafts, the anesthetized animals were placed in a small amount of oxygenated water for recovery. The animals were so placed that the operated areas were exposed to the air, which condition tended to hasten the congealing of the lymph and blood which serve to hold the graft in place until definite

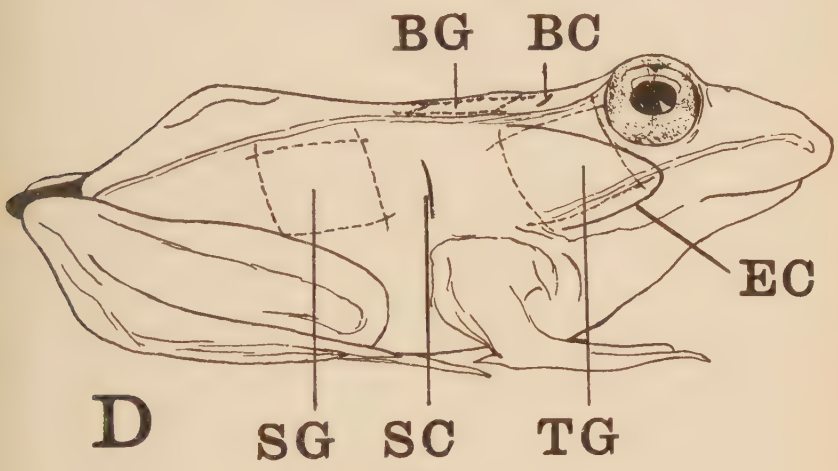


FIG. D

healing has occurred. Upon recovery, the animals were immersed in water, fed *Spirogyra*, and allowed to metamorphose.

3. SERIES APT

This series consisted of the reciprocal transplantation between skin grafts of the side and tympanic membrane region (*SG*, *TG*, Fig. D). The metamorphic stage operated on varied considerably and covered a range from the stage immediately following fore-limb eruption to one near the end of metamorphosis when the tail had been resorbed to a small black stump. The grafts taken from the tympanic membrane regions of the tadpoles in the earlier stages of transformation were in very early histological stages of tympanic membrane formation. Those taken from later stages were considerably more advanced, histologically, and presented fairly distinct pictures, macroscopically, of tympanic membrane formation. The

side grafts transplanted to the tympanic membrane region, however, represented normal integument in all cases.

Within three days following transplantation, the grafts were usually fully healed, the fusion area being represented by a narrow black line. The various steps taken during this healing process have been described by the writer in a previous paper (1926) and will not be described here. The reader is also referred, in this connection, to the works of Peters (1885), Barfurth (1891), Loeb (1898, 1902), Harrison (1910, 1914), Holmes (1913, 1914), Oppel (1913), Cole (1922), *et al.*

Following the healing period, no definite visible changes were observed for from 3 to 5 days, during which time the tadpoles were rapidly completing the last stages of metamorphosis. At that time, however, distinct pigmentary changes occurred in the grafts transplanted to the tympanic membrane region, which were typical of normal tympanic membrane formation. These were shortly followed by a perceptible outlining of the area exhibiting pigmentary changes. The area so outlined was similar in shape, size, and orientation to that of a normal tympanic membrane. In a few cases more or less translucent, crescent-shaped areas formed at the periphery of the areas, usually on the ventral boundary. Further modifications of these areas consisted chiefly of a gradual change in pigmentation, during which a brownish coloration was assumed similar to that possessed by normal tympanic membranes. The areas also presented slight indentation in general. Failure of the animals to live longer than 10–14 days following the operations, however, prevented the further development of tympanic membrane characteristics. The characteristic appearance of the grafts just prior to the death of the animals is represented in Figures 17 and 18, Plate V.

Several of the grafts were sectioned and examined, microscopically, for possible histological signs of tympanic membrane formation. In all cases where external changes had been recorded, definite transformations had occurred which were typical of normal histological membrane development (Fig. 7, Plate II). It was noted in particular that the portions of the graft which had been in contact with the rim of the annular tympanic cartilage were especially well

advanced in this transformation. The stratum spongiosum at such points was obliterated, while the stratum compactum presented the typical granular and fibrous condition as found in the fully developed tympanic membrane. The presence of lymphocytes in considerable abundance and the dissociated appearance of the stratum compactum of the central regions further emphasized the conclusion that definite histological transformations, identical with those of normal tympanic membrane formation, were in operation.

The fate of the reciprocal, autoplastic grafts transplanted from the tympanic membrane region to the side presented little or no external modifications. Following the healing period, the only marked visible tendency was to assume, in general, the same depth of pigmentation as presented by the surrounding integument. Histological sections of such grafts showed quite clearly that the various layers of the integument were rapidly assuming a normal structure. Grafts that had been transplanted when in early stages of tympanic membrane formation presented the histological appearance of normal integument. In other words, the layers of the integument which had undergone slight histological changes shortly retrogressed to the condition of normal integument upon transplantation. In the case of grafts transplanted during later stages of tympanic membrane formation, the retrogression was not so complete, since more pronounced changes had taken place prior to transplantation. In general, it was clear that such integument never tended to complete the histological tympanic membrane changes present when removed from the tympanic membrane region, but on the contrary, always underwent transformations directed to establish the normal integumentary picture.

4. SERIES ABT

Series ABT animals involved the same type of operation as employed for Series APT individuals, except that grafts of skin from the back were interchanged with those of the tympanic membrane region (*BG*, *TG*, Fig. D). The individuals used were generally in somewhat later stages of metamorphosis than those of the preceding series, since the best results of the latter group had been obtained with such forms. The average specimens employed were nearing the end of metamorphosis, the tail being reduced to a stump, and the

tympanic membranes appearing quite distinct as to shape and texture. The operation, therefore, really consisted of removing a square of skin containing the half-formed normal tympanic membrane and interchanging it with a square of normal integument of the back.

The back-skin grafts, so transplanted, were deeply pigmented and presented a uniform black coloration. Within 4 days, on the average, the grafts were fully healed and assumed a much lighter, brownish shade. In a few cases the earliest signs of tympanic membrane formation appeared on the sixth day following transplantation to the tympanic membrane region. The average time required for all cases was about 9 days, however. Following this, the usual macroscopic developments of tympanic membrane formation occurred in logical order. It was at once apparent that development was proceeding to a far greater degree in these individuals than in those of the earlier series. This will be evident by reference to Figure 13, Plate IV, which represents the typical condition of the developing tympanic membrane when death occurred on the twelfth to fifteenth day following transplantation. The tympanic membranes, so formed, were similar in all respects to normal membranes as developed on the opposite sides of the same individuals (Fig. 11, Plate IV). In general, it was noted, however, that the degree of development was always somewhat behind that of the normal left-side membranes—a condition to be expected in view of the comparatively shorter period of time available for right-side membrane development.

Microscopic examination of the membranes so developed revealed, as in the preceding series, the typical cellular modifications of normal membrane formation. The extent of the histological transformations, however, was in all cases considerably more advanced and consistent with the degree of development as evidenced by the external stage of membrane formation. Figure 6, Plate II, represents the typical condition found. It will be noticed here that all but a small central area of the membrane presents the cytological conditions typical of a fully formed membrane. Evidently this is the last portion of the membrane to complete its development. The reappearance of the stratum spongiosum with scattered glands and

the relative scarcity of lymphocytes further emphasize the late stage of membrane formation present.

The reciprocal, tympanic membrane grafts, transplanted to the back, underwent similar retrogressive transformations as already described for grafts transplanted to the side. Externally, the grafts tended to assume the darker pigmentation of back skin while the half-formed tympanic membranes became less conspicuous and finally lost their characteristic, brownish coloration and texture. The area could always be identified, however, due to the persistence of a certain amount of indentation. Histologically, the grafts again presented definite signs of retrogression and a decided transformation back toward the conditions typical of normal integument.

ANNULAR TYMPANIC CARTILAGE TRANSPLANTATIONS

1. PURPOSE OF THE CARTILAGE TRANSPLANTATIONS

The results of the reciprocal grafting of back and side skin with skin of the tympanic membrane region showed quite clearly that the skin of the latter is not peculiarly, hereditarily specific toward tympanic membrane formation. When transplanted to foreign locations, it fails to develop membranes during metamorphosis; while at the same time back and side skin transplanted to the tympanic membrane region transform into normal tympanic membranes. Such results lead one, therefore, to the conclusion that structures peculiar to the tympanic membrane region must be responsible in producing the transformations induced in the integument of that region during metamorphosis.

The most obvious structure in this region, and the only one in contact with the integument, is that of the annular tympanic cartilage. The possibility that it exercises a developmental influence on the formation of the tympanic membrane is strengthened when we consider its parallel development with that of the latter. It is always the skin directly over the cartilage which transforms into membrane. Moreover, the size, shape, and orientation of the membrane is identical with that of the annular tympanic cartilage.

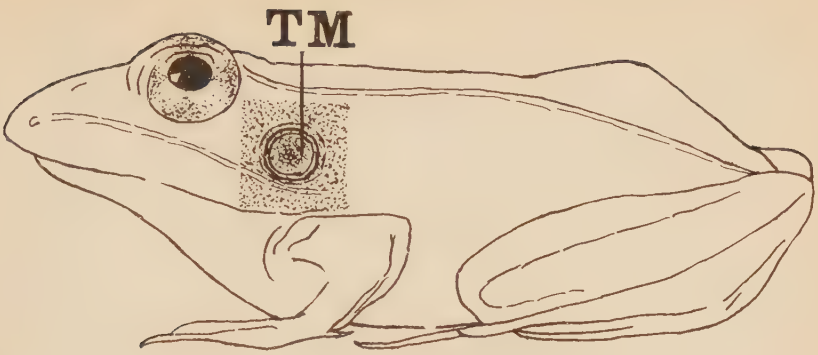
Obviously, the extirpation and transplantation of the cartilage at various stages of development should throw light on this ques-

PLATE IV

FIG. 11.—Normal control tympanic membrane as developed on the left (unoperated) side of individuals whose right annular tympanic cartilage had been extirpated. *TM*, tympanic membrane. ×4.

FIG. 12.—Sketch showing absence of tympanic membrane formation during metamorphosis following previous extirpation of the annular tympanic cartilage. *EC*, scar of extirpation cut made when removing annular tympanic cartilage. ×4.

FIG. 13.—Formation of a tympanic membrane in a back-skin graft previously transplanted to the tympanic membrane region. *BG*, back-skin graft; *TG*, skin graft from tympanic membrane region transplanted to the back; *TM*, tympanic membrane. (Case ABT 30.) ×4.



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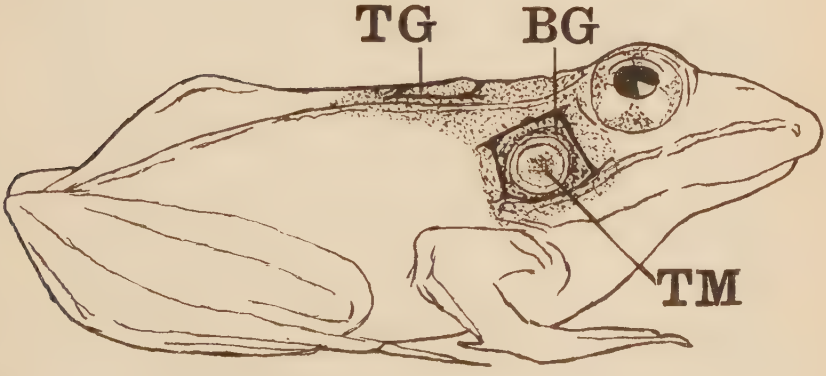

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tion. If the cartilage normally exerts such an influence, then its extirpation at an early stage should inhibit the development of a tympanic membrane, either partially or wholly, depending on the degree of influence. Furthermore, should tympanic membranes fail to develop in the absence of the cartilage, it should be possible to form tympanic membranes in any integument by the transplantation of the cartilage beneath and in contact with such skin. Operations were consequently designed to investigate such points.

2. TECHNIQUE OF CARTILAGE EXTIRPATION AND TRANSPLANTATION

The technique employed in extirpating the annular tympanic cartilage was subject to some variation depending on the stage during which the cartilage was removed. It will be remembered in this regard that the developing ring of cartilage undergoes a gradual migration anterioposteriorly during metamorphosis. In general, however, the following method was employed: The tadpoles were anesthetized, and a flap of skin (*EC*, Fig. D) was carefully cut and laid back in order to expose the developing cartilage. In this regard it was necessary to avoid cutting the skin at any point destined to transform into tympanic membrane during normal metamorphosis. The ring of cartilage was then carefully separated at the periphery from adjoining tissues and severed from its attachment by snipping with a fine-pointed pair of scissors. By using considerable care, it was possible in this way to remove the ring of cartilage at any stage of development with a minimum of bleeding. The prevention of excessive bleeding is very important in metamorphosing tadpoles, since they are generally quite weak during this period and fail to recover following loss of much blood. Following the removal of the cartilage, the flap of skin was then carefully replaced and the cut edges smoothed out to facilitate healing. The next step consisted in transplanting the ring of cartilage beneath either side-skin or back-skin. This was accomplished in the following manner: A small cut just sufficient to allow of the introduction of the cartilage was made in the skin at a point considerably anterior to the desired orientation of the transplant. The extirpated ring of cartilage was then pushed through this opening by means of a small spear-head scalpel and forced back under the skin. In this regard it was neces-

sary first carefully to loosen the skin from the underlying peritoneum of the side or muscles of the back, as the case might be. Care was also exercised to have the lateral side of the cartilaginous ring facing and in contact with the skin, in order to duplicate the normal orientation as found in the tympanic membrane region. The operated animals were then placed on their sides in shallow water, to allow adhesion of the flap of skin over the extirpation area, and allowed to recover from the anesthetic.

3. SERIES ATC

This series consisted of the extirpation of the annular tympanic cartilage in various stages of development and its autoplastic transplantation beneath the skin of the side. The stage operated on varied from one in which the tail was but half-atrophied and only the earliest external signs of tympanic membrane were visible, to one near the close of metamorphosis when the tail was reduced to a mere stump and the tympanic membrane was about half-formed. The annular tympanic cartilages of the early stages were quite small, at which time they are found developing ventrally and somewhat anteriorly to the eye (*ATC*, Fig. A). Those removed from animals in the later stages of metamorphosis were comparatively much larger and circular in shape and occupied a more posterior location (*ATC*, Fig. B). Transplantations of the annular tympanic cartilage were made at all intervening stages between these two extremes.

The effect on the skin of the side in the region of the cartilage transplants varied considerably according to the stage of the annular tympanic cartilage transplanted. In the case of the early-stage transplants, only slight or no external change was observable. The integument generally appeared quite normal and gave no evidence, either through coloration or bulging, of the presence of the cartilage. Subsequent examinations of such individuals showed that the transplants in most cases had failed to thrive in their new location, and, while a few were attached to the under side of the integument, practically none had established connections with the peritoneum. The small size of such transplants, furthermore, made strong contact with the integument impossible. It was quite evi-

dent that a good proportion of the transplants had undergone slight disintegration, and in no case had growth taken place. Sections made through the integument in contact with the cartilage gave little or no evidence of tympanic membrane changes.

Pronounced macroscopic and microscopic changes were found to occur in the side skin where the larger and later-stage transplants were employed. Here, a distinct though slight bulging of the skin occurred, due to pressure of the transplant beneath; and the area not infrequently assumed a light-brownish coloration, indicative of early tympanic membrane formation. Distinct outlining occurred in several cases, which coincided with the rim of the cartilage transplants beneath. The outlining was evidenced by a pronounced grayish translucency of the skin (*ATC*, Figs. 15 and 16, Plate V) and was present only when, as subsequent examinations showed, the integument was in strong contact with the transplant.

Histological sections made through such integument showed that typical histological tympanic membrane transformations were well under way (Fig. 8, Plate II). It was also clear from the sections that the degree of tympanic membrane formation was in direct proportion to the degree of contact of the skin with the transplants. It was furthermore noted that the time element played an important rôle, in that individuals surviving for longer periods of time presented the most pronounced signs of tympanic membrane formation. The logical explanation for this is that the transplants in such cases had a longer time in which to influence the development of cytological changes in the integument.

The extirpation of the annular tympanic cartilage prevented the development of tympanic membranes in the normal location in all cases. When the transplants had been removed during an early stage of development, the integument of the tympanic membrane region remained normal throughout metamorphosis and resembled in all respects, both visibly and histologically, normal skin of the neck (Fig. 12, Plate IV). When the transplant had been extirpated at a late stage of development and not until the normal tympanic membrane had been half-formed, there was a decided tendency for the membranes to retrogress and assume the external and microscopic characteristics of normal integument. In either case, the

results indicated quite conclusively that the annular tympanic cartilage was essential for membrane development. In this regard it may be said that the tympanic membranes as developed on the left and unoperated side (Fig. 11, Plate IV) were always well formed and served as controls for all extirpations made on the right side.

4. SERIES CB

Series CB operations resembled the transplantations as described above in all respects except that the transplants were made beneath the skin of the back instead of beneath side skin. The transplants when extirpated varied as regards their degree of development, although the majority were in late stages of formation, many having attained the size indicated in Figure B, *ATC*. Because of their relatively large size, they were always in strong contact with the integument when transplanted, and produced a strong bulging of the latter.

The first macroscopic signs of tympanic membrane formation occurred within 4–5 days following transplantation. Unfortunately, however, owing to the late metamorphic stage of the individuals operated on, no cases survived longer than 5 days following this period. The time available, however, was sufficient for the formation of several remarkably well developed membranes, which were identical in all respects to normal tympanic membranes (*TM*, Fig. 14, Plate V). The development in certain cases was so perfect that the finer, characteristic color shadings and peculiar texture of the membrane were fully developed.

Several serial sections were made through such membranes, the sectioning including the cartilage transplants beneath (Fig. 10, Plate III). Such sections revealed remarkably identical histological pictures when compared to similar sections made through normal tympanic membranes of the head region, as pictured in Fig. 9, Plate III. The similarity not only included the various layers of the integumentary portion of the tympanic membrane but also the lamina propria and internal epithelial layers. It was also evident that the cartilage transplants were in a healthy condition and their cells undergoing normal division. The presence of a supporting, loose connective tissue containing scattered lymphocytes and other

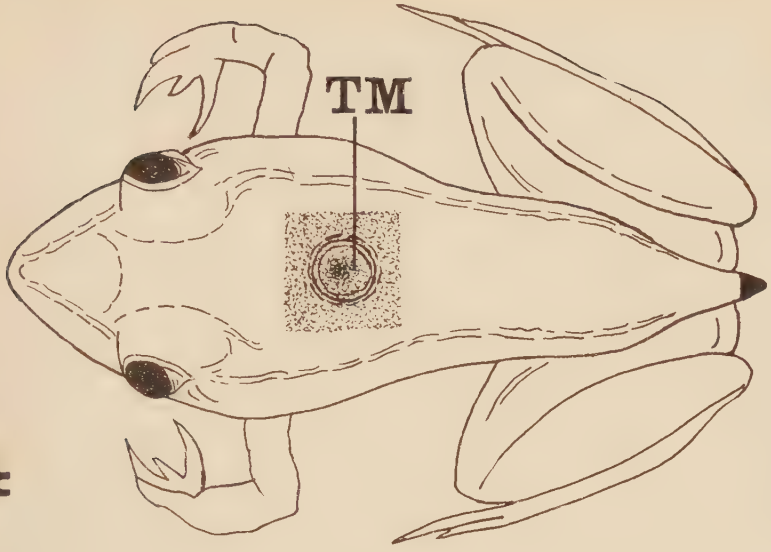
PLATE V

FIG. 14.—Formation of a tympanic membrane in skin of the back underneath which an annular tympanic cartilage had previously been transplanted. *TM*, tympanic membrane. (Case CB 15.) ×4.

FIGS. 15, 16.—Partial tympanic membranes formed in skin of the side underneath which annular tympanic cartilages had previously been transplanted. *TM*, tympanic membrane; *ATC*, outlining of membrane, indicating position of annular tympanic cartilage transplants. (Cases ATC 29 and 19, respectively.) ×6.

FIGS. 17, 18.—Partial tympanic membranes formed in side-skin grafts previously transplanted to the tympanic membrane region. *SG*, side-skin grafts; *ATC*, translucent areas indicating presence of annular tympanic cartilage beneath skin; *O*, outlining of developing tympanic membrane indicating location of annular tympanic cartilage. (Cases APT 6 and 15, respectively.) ×6.

PLATE V



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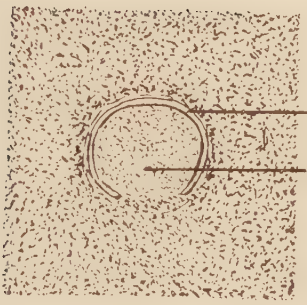

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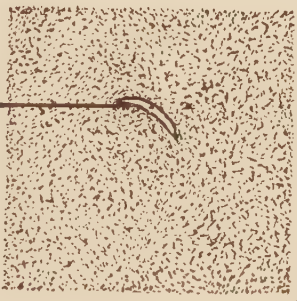

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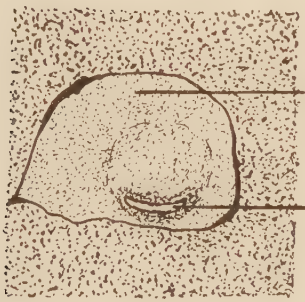

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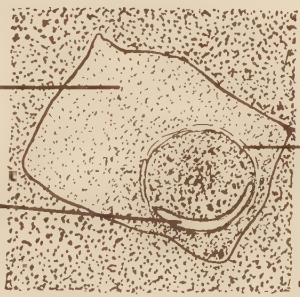

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blood cells completed a picture indistinguishable from that of the normal membrane and adjacent structures.

DISCUSSION

The formation of the tympanic membrane presents many histological structures of interest, especially as regards their morphological and embryological nature. The replacement of the stratum compactum by a fibrous layer is perhaps the most noteworthy histological transformation. That this layer is really structurally different from the compactum layer of normal integument seems quite evident to the writer. That two distinct sets of fibers are present is also evident and in agreement with the findings of Tokura (1925). The mode of their origin, however, is quite problematic. The apparent breaking up of the normal compactum layer and its invasion by lymphocytes would suggest that the layer is completely broken down and removed. If this be true, the fibers of the new compactum layer of the future tympanic membrane must be formed from other connective tissue elements which make their way to this region at this time. The fact, however, that the formation of the fibers and the breaking down of the old compactum layer appear to occur simultaneously during the late stages of the disruption of the layer would suggest that the fibers may possibly be formed from dissociated elements. Certainly it is true that by the time phagocytic action is on the wane, the fibers with their characteristic granulated appearance can be observed and identified from the dissociated remnants of the compactum layer. Furthermore, the gradual merging of this fibrous layer with the stratum compactum of the surrounding integument would also suggest such a developmental transformation.

The results of the skin-transplantation experiments (Series APT and ABT) have emphasized the fact that the integument of the tympanic membrane region is not a self-differentiating tissue as regards its ability to transform into tympanic membrane during metamorphosis. It is apparent that in this physiological respect, at least, all integument possesses like potentialities, in that foreign skin is capable of tympanic membrane formation when in the tympanic membrane region. This same tendency of integument to

react alike, physiologically, has also been demonstrated by the writer (1926) in connection with the formation of the opercular fore-leg perforations. It was found in this connection that integument of the back and side, when transplanted to the region of fore-limb eruption, underwent typical histolysis which resulted finally in the appearance of a normal perforation. In the present problem, the fact that integument of the tympanic membrane region when transplanted to the back or side failed to develop into tympanic membrane during metamorphosis, further emphasizes the conclusion that such integument is not hereditarily specific for membrane formation, but undergoes such transformations owing solely to its association with structures of the middle ear.

The experiments involving extirpation and transplantation of the annular tympanic cartilage indicate quite conclusively that this structure constitutes the immediate influence responsible for the development of the tympanic membrane. It is also quite evident that it is the only causal agent concerned, since removal of the cartilage inhibited the development of even the earliest signs of membrane formation. Conversely, the membranes formed in foreign integument under which the cartilage had been transplanted, were typical in all respects, including the specific histological developments and the formation finally of well-formed membranes.

The period of development of the annular tympanic cartilage during which it is physiologically active in membrane formation is an important point. From the results of the cartilage-transplantation experiments, it would appear that it possesses such an influence from a very early stage of development until the stage reached at the close of metamorphosis. This physiological influence, although very slight at first, gradually increases, and reaches a maximum slightly before metamorphosis is completed. It is interesting to note here, that although the cartilage is physiologically active at a very early developmental stage, it apparently exercises no influence toward the formation of the normal tympanic membrane at that time. This is undoubtedly due to the fact that the cartilage is not in contact with the integument at the time but is undergoing a posterior migration. It is only when the cartilage reaches the location typical of membrane formation that it is brought into firm

contact with the integument, to which it shortly adheres. It is evident, then, that although the cartilage is physiologically active as regards membrane formation at a very early developmental stage, it cannot exercise this power until its migratory development has brought it in strong contact with the integument.

The fact that actual contact of the cartilage with the integument is essential before histological tympanic membrane changes may occur in the latter, naturally raises the question regarding the mode of influence in operation. It is just possible that the pressure exerted against the integument by the cartilage is sufficient partially to occlude the blood supply to the former. This, in time, would produce a condition of acidosis resulting from the failure of the blood stream adequately to remove products of excretion by the tissue, and in this manner initiate the histolytic processes typical of the early stages of membrane formation. It is difficult to see, however, how a condition of acidosis capable of producing histolysis could be responsible for the formative transformations involved during the later stages of membrane formation. A more logical assumption, according to the writer, would be to assume the action of a specific secretion or hormone of the annular tympanic cartilage. Such a secretion or hormone, applied by direct contact to the integument, could function as the causative agent of histolysis at first, and later as a stimulus to the development of the structural components of the adult membrane. It is also quite logical to assume that two hormones may be concerned—one responsible for the early histolytical changes, and the other for the later formative developments.

Regardless of the exact nature of the physiological influence exerted, it is clear that the influence not only requires direct contact with the skin to become active but requires a considerable period of time to bring about the development of a complete tympanic membrane. If, for example, the annular tympanic cartilage is extirpated at a time when the membrane is forming, the result is to inhibit all further tympanic membrane developments. In fact, there is a decided tendency for the incompletely formed structures to undergo histological changes designed to re-establish the structure of normal integument. This was evident not only in many cases of cartilage extirpation but also in all cases where developing

membranes were transplanted to the back or side and therefore away from the influence of the cartilage. The fact that considerable enlargement of the tympanic membrane takes place following metamorphosis, during the growth of the young frog, would suggest, strongly, that the annular tympanic cartilage continues to exert its influence on membrane formation during this period also.

The mechanism of tympanic membrane formation, finally, serves as a good example of what might be called the "indirect action" of the thyroid hormone in amphibian metamorphosis. It is the thyroid hormone which primarily sets in operation certain fundamental reactions, which in turn bring about the development, transformation, or degeneration of other structures which in themselves may then effect the development or destruction of still other structures. Thus it is possible for the thyroid hormone to set in motion a series of interrelated reactions which finally bring about an end result. Some of the reactions concerned in the formation of the tympanic membrane of the frog would therefore appear to be as follows: The thyroid hormone either directly or indirectly initiates influences which bring about certain transformations of the skeletal system during metamorphosis. One of these transformations results in the development of the annular tympanic cartilage from the metapterygoid region of the quadrate. The annular tympanic cartilage during its growth generates an influence, probably hormonic in nature, which, when applied by contact to integument, initiates histological transformations resulting in the formation of a tympanic membrane.

GENERAL SUMMARY AND CONCLUSIONS

1. The formation of the tympanic membrane in *R. pipiens* is the result, histologically, of a definite, orderly process involving the histolysis and reconstruction of certain integumentary layers. The appearance of numerous, invading lymphocytes is a secondary and phagocytic phenomenon and is not to be regarded as causing the histolysis.

2. Autoplastic skin grafts from the back and side were interchanged with skin grafts from the tympanic membrane region, and the tadpoles were allowed to metamorphose. Typical tympanic

membranes formed in the grafts transplanted to the tympanic membrane region. The integument of the tympanic membrane region, when transplanted to the back or side, failed to develop membranes.

3. The results of skin transplantation indicate that all integument is capable of transforming into tympanic membrane when in the tympanic membrane region. The normal integument of that area is not, therefore, a self-differentiating and hereditarily specific tissue.

4. The annular tympanic cartilage was extirpated at various stages of development, and the individuals metamorphosed. No cases of membrane formation occurred following extirpation of cartilages in early developmental stages. Extirpation of cartilages, when the membrane had begun to form, prevented further development of membranes. The membranes subsequently tended to transform back into normal integument.

5. Annular tympanic cartilages were transplanted, autoplastically, in various stages of development beneath the skin of the side and back. Tympanic membranes were formed in such locations directly over the transplants. The completeness of the membranes formed was dependent on the developmental stage of the cartilage transplanted, the length of time the skin was in contact with the transplant, and the degree of contact existing between transplant and skin.

6. The conclusion is reached that the histological transformations in the integument during tympanic membrane formation are the result of influences exerted by the developing annular tympanic cartilage. The suggested influence is that of a secretion or hormone applied by direct contact to the integument by the cartilage.

7. The mechanism of tympanic membrane formation illustrates an indirect method of the thyroid hormone in bringing about certain metamorphic transformations. In this case it would appear that the thyroid hormone either directly or indirectly stimulates the metapterygoid region of the quadrate to form the annular tympanic cartilage, which, as development progresses, liberates an influence, probably hormonic in character, whose application to the integument induces the histological transformations essential for tympanic membrane formation.

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The Relief of Parathyroid Tetany by Injections of Uranium Nitrate

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THE RELIEF OF PARATHYROID TETANY BY INJECTIONS OF URANIUM NITRATE

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RECENT experimental studies have indicated that slight changes in the acid-base equilibrium, with a shift in the reaction of the blood toward the acid side, exerts a profound effect upon the tetany of parathyroid insufficiency. For example, Wilson, Stearns, and Janney (1915) report that injection of 0.1–0.5 gram of hydrochloric acid promptly brings relief to dogs suffering from severe tetany. The work of these investigators was followed by that of György (1922), Gamble and Ross (1923), and Anderson and Graham (1924), who were able to demonstrate that acid-producing salts such as calcium chloride and ammonium chloride are highly effective agents in the treatment of infantile tetany and, when given in sufficient quantity, relieve all symptoms.

Boyd, Austin, and Ducey (1926), and Wenner (1927) working independently, repeated the experiments of György, and Gamble and Ross, using parathyroidectomized dogs as experimental animals. It will be recalled that parathyroid removal when practiced on dogs is almost invariably fatal, and the tetany symptoms are far more severe than those usually observed in infants. However, ammonium-chloride administration brings about a prompt cessation of symptoms, even the most violent, within a short time, and the animals return to an apparently normal state. The serum calcium of dogs so treated generally, though not invariably, shows a slight increase, but never rises to the preoperative level normal for this species, i e., 10–11 mgm. per 100 cc. of blood.

These observations indicate that the administration of NH_4Cl, by increasing HCl metabolism and decreasing the pH of the blood, somehow influences the solublity of calcium, thereby increasing the amount of this substance in the circulating fluids of the body and bringing about relief from tetany. It is fast becoming obvious that it is not the quantity of total calcium in the blood and tissues which

is important in tetany prevention and relief, but the solubility of certain types of calcium compounds. This point will be more fully discussed later.

Swingle, Wenner, and Stanley (1928) demonstrated that partial asphyxiation of parathyroidectomized dogs in violent tetany by CO_2 promptly relieves symptoms. The animals return to normal and remain free from symptoms for some hours. Dogs treated with CO_2 exhibit marked acid intoxication, as shown by lowered CO_2 capacity, CO_2 content, and pH of the blood. It was demonstrated that the acidosis was not due to CO_2 but to rise in lactic acid due to anoxemia. When the blood lactic acid returned to the normal level (approximately 28 mgm. per 100 cc.), tetany recurred. Despite the fact that the CO_2-treated dogs appeared free from tetany, the serum calcium remained low and was not markedly affected by the CO_2 treatment. A slight rise in calcium invariably occurred, but in several instances was within range of the experimental error of the method employed for its determination. It is our opinion, however, that the relief from tetany which followed the use of CO_2 was due to the increased solubility of certain types of calcium compounds at the lowered pH.

Greenwald and Gross (1925 *a*, *b*) observed, in their study of the pathogenesis of tetany, that following parathyroidectomy there is no increased excretion of calcium, as reported by Salvesen (1923), but an actual decrease in the elimination of both calcium and phosphorus. Whereas, after injection of Collip's active parathyroid extract, increased excretion of both calcium and phosphorus occurred. They suggest, on the basis of their observations, that after parathyroidectomy, calcium is precipitated in the tissues as calcium phosphate, which in the absence of the parathyroid hormone becomes less soluble. In the light of their observations, it seemed likely that a slight shift in the reaction of the blood toward the acid side increases the solubility of certain calcium compounds, and that any substance inducing such a change in reaction would prove effective in the treatment of parathyroid tetany.

During the course of certain acid-intoxication experiments on dogs which involved the use of nephrotoxic substances such as uranium nitrate, the idea occurred that this drug should be useful

in controlling and relieving tetany because of the marked change in the reaction of the blood toward the acid side which follows its administration. It has been known for years that following subcutaneous injections of uranium nitrate, a marked acid intoxication slowly develops which is likely to prove fatal if large doses of the drug are used. The work of MacNider (1920) and others showed that uranium nitrate is a powerful nephrotoxic drug and induces severe degenerative changes in the tubular epithelium of the kidneys. The nature of the acid or acids which are responsible for the acidosis following its use is not known for certain.

The idea which most readily comes to mind with regard to the cause of the acidosis of uranium-nitrate poisoning is that since this substance is nephrotoxic and causes kidney lesions, the acid-eliminating functions of the kidneys are interfered with to such an extent that acid substances formed in the course of metabolism and normally eliminated by the kidneys are retained. These substances accumulate in the blood and eventually induce the type of acid intoxication characteristic of the terminal stages of uremia. Although the supposition seems logical enough and is commonly accepted as an adequate explanation, it nevertheless does not fit the facts when subjected to scrutiny, for the following reasons:

1. According to MacNider, who has worked extensively with uranium nitrate, the acidosis which develops following its injection into dogs may appear before degenerative changes in the kidneys become evident.

2. Atchley and Benedict (1927) and Swingle, Wenner, and Stanley (1928) have demonstrated that following bilateral ureter ligation and total kidney extirpation, acid intoxication, if present at all, in such operated animals is not very marked.

3. Wallace and Pellini (1921) report the case of a dog from which both kidneys had been removed, which showed no acidosis. However, when the animal was injected with uranium nitrate, severe acid intoxication developed.

In the experiments reported here, our chief interest was in the effect the acidosis of uranium-nitrate poisoning might have upon the relief of parathyroid tetany, but an attempt was also made to determine the nature of the acid or acids responsible for the intoxication itself.

Ten dogs were employed in the experiments. They were of various ages and sizes, complete data concerning some of them are given in the protocols. The animals were parathyroidectomized and allowed to develop marked tetany symptoms. They were then bled for CO_2 capacity, pH, and serum calcium. It has been the experience of those working in this laboratory, Wenner and Muntwyler (1927), and others, that the CO_2 capacity and pH of tetany dogs are normal; and our own personal experience corroborates this view, except when a dog has just recovered from a series of violent convulsions, for in such cases one finds a lowered blood pH and CO_2 capacity, due to rise in lactic acid. Omitting such exceptional cases however, the blood of tetany animals is normal in so far as any evidence of acidosis or alkalosis is concerned.

Following bleeding, the tetany animals were injected with uranium nitrate subcutaneously. The dosage depended upon the age of the animal, in so far as we were able to determine the age of our mongrel dogs, for, as MacNider has emphasized, old dogs are much more susceptible to uranium nitrate than are younger animals. However, none of the dogs received more than 6 mgm. per kilo.

The effect of uranium nitrate upon parathyroidectomized dogs presenting violent tetany symptoms is indeed striking, for within 12–24 hours after injection, all manifest tetany symptoms have disappeared and the animals run about, eat, play, and act in normal fashion. A dog may be prostrate with tetany, unable to walk or even stand erect, yet within a short time following injection, the same animal presents no signs of tetany, and indeed differs in no way from an unoperated dog. The normal condition may last a week, 10 days, or even 2 weeks, before tetany again develops. Meanwhile the animal has been receiving a heavy meat diet. When tetany again appears, another injection of uranium nitrate is necessary, following which the tetany symptoms disappear; but generally the second injection is followed within a few days by symptoms of uranium poisoning which soon leads to death. No attempt was made to keep the animals alive indefinitely by employing very small doses of the drug when tetany recurred, although it is probable that this could be done. Complete data on two of our ten experimental animals are given in the protocols.

PROTOCOL 1

Female fox terrier. Weight, 8 kilos. Bled from heart, February 21. CO_2 capacity, 45.4 vols. per cent. pH, 7.38. Serum Ca, 13 mgm. Thyroparathyroidectomized February 13, 10:00 A.M. February 27, 9:00 A.M., dog in tetany. Muscular twitching and jerkings; animal spastic and refuses food. Bled. CO_2 capacity, 45.5 vols. per cent. pH, 7.37. Serum Ca, 7 mgm. per 100 cc. After bleeding, dog given 40 mgm. uranium nitrate subcutaneously, or about 5 mgm. per kilo of body weight.

February 28. Tetany symptoms absent; dog seems normal. Eats and plays.

March 2. Animal normal. Bled. CO_2 capacity, 21.1. pH, 7.23. Ca, 7.5 mgm.

March 3, 4, 5, and 6. Animal remained normal. Evening of the 6th, the animal seems listless and eats little. Shows symptoms of uranium poisoning.

March 7. Bled. CO_2 capacity, 19.8 vols. per cent. pH, 7.22. Ca, 7.2. Animal shows symptoms of severe acidosis, but no tetany.

March 8. Animal died of uranium-nitrate poisoning.

PROTOCOL 2

Mongrel pup, male. Weight, 7.5 kilos. Operated February 6, 2:00 P.M.

February 10. Tetany present. Animal very spastic; marked tremors and jerking of limbs. Salivating and panting. Bled. CO_2 capacity, 38.9 vols. per cent. pH, 3.34. Serum Ca, 7 mgm. per 100 cc. Dog injected at 10:00 A.M. with 45 mgm. uranium nitrate, or about 6 mgm. per kilo of body weight. Five P.M. of the same day animal ate meat and presented no tetany symptoms.

February 10–18, dog remained normal. February 18, dog bled. CO_2 capacity, 37.3. pH, 7.36. Ca, 8.2 mgm.

February 19–21. Animal perfectly normal. Bled February 21. CO_2 capacity, 28.3 vols. per cent. pH, 7.34. Ca, 8 mgm.

February 21–27. Animal free from tetany. Bled. February 27. CO_2 capacity, 35.3 vols. per cent. pH. 7.34. Ca, 8.5 mgm.

February 28—March 1. Animal normal. March 1, 3:00 P.M. Slight tetany symptoms apparent. Bled. CO_2 capacity, 35.5. pH,

7.35. Ca, 7 mgm. Animal given 3 mgm. uranium nitrate per kilo body weight.

March 2. Dog sleepy and listless; no tetany. March 3–8. Animal lethargic and apparently suffering from uranium-nitrate poisoning. Tetany symptoms absent.

March 9. Dog died of uranium-nitrate poisoning.

Table I gives in abbreviated form the data obtained from six of our nine cases. The remaining three animals behaved similarily to uranium-nitrate treatment, but were not bled so frequently for study of the acid-base equilibrium at various stages of the experiment.

Analysis of the data in Table I reveals several interesting facts. It is quite evident that the injected animals develop a severe acid intoxication following injection of uranium nitrate; the CO_2 capacity and pH drop markedly, and in most of our cases death eventually occurred from uranium-nitrate poisoning. The intoxication develops rather slowly and appears to be much more severe in some animals than in others. This variation is in all probability due to differences in the ages of the animals, since MacNider (1920) has clearly shown that old dogs react much more violently to uranium nitrate than do young ones. In fact, doses of the drug that are lethal for old animals may cause but transitory symptoms of acid intoxication in younger dogs.

Another point of interest is the rise in serum calcium which follows injection of uranium nitrate. While the increase is small, it invariably occurs; and in two cases it amounted to more than a milligram of calcium per 100 cc. of blood. This was true of animals No. 1, 2, and 4. Recently the writer and his collaborators (1928) reported similar small increases in the serum calcium following CO_2 administration to tetany animals. Wenner (1927) likewise reported increases after large doses of NH_4Cl. In all cases the increase in serum calcium which was noted appeared to be correlated with the fall in CO_2 capacity and pH. A slight shift in the reaction of the blood toward the acid side not only relieves tetany but induces a slight rise in the total calcium of the serum. This increase is not lasting, for the calcium may again decrease to levels lower than that observed when the tetany symptoms were first noted. For an example of this, note the data concerning dog 7 in Table I.

TABLE I

EFFECT OF URANIUM NITRATE ON PARATHYROID TETANY

Dog	Operated	Tetany	Bled	CO_2 Cap. Vols. per Cent	pH	Ca Mgm. per 100 Cc.	Remarks
1.	Jan. 27	Jan. 28	Jan. 28	40.7	7.38	6.2	Tetany. Injected 10 mgm. (2 per kilo) uranium nitrate 10:00 A.M. Jan. 28. Normal; ate and drank.
			Jan. 30	36.8	7.37	7.2	Condition fair. No tetany.
			Feb. 3	36.4	7.35	7.8	Ate. Injected 6 mgm. (1.2 per kilo) uranium nitrate.
			Feb. 4	29.8	7.25	5.8	No tetany. Injected 6 mgm. (1.2 per kilo) uranium nitrate. Died.
2.	Jan. 27	Jan. 30	Jan. 30	39.7	7.37	6.6	Slight tetany. Injected 78 mgm. (6 per kilo) uranium nitrate 9:30 A.M.
			Jan. 31	34.2	7.35	6.9	Animal normal.
			Feb. 2	32.4	7.33	7.9	Animal normal.
			Feb. 14	30.1	7.30	7.2	Refused food. Injected 65 mgm. (5 per kilo) uranium nitrate. Uranium poisoning.
4.	Feb. 6	Feb. 10	Feb. 10	38.9	7.34	7	Tetany. Injected (6 mgm. per kilo) uranium nitrate 10:00 A.M. Feb. 10. Recovery after 24 hours. Heavy meat diet.
			Feb. 12	37.3	7.36	8.2	Seems normal.
			Feb. 20	28.3	7.34	8.0	Animal normal.
			Feb. 27	35.3	7.34	8.0	Condition excellent.
			Mar. 1	35.5	7.35	7.0	Tetany. Refused food. Given 3 mgm. uranium nitrate per kilo.
			Mar. 8	24.2	7.27	6.8	No tetany. Symptoms acidosis.
			Mar. 10				Died uranium-nitrate poisoning.
7.	Feb. 11	Feb. 13	Feb. 11	47.1	7.39	8.0	Tetany, given 5 mgm. uranium nitrate per kilo. Normal after 36 hours. Eats meat and runs about.
			Feb. 15	40.9	7.35	7.1	No tetany symptoms. Heavy meat diet.
			Feb. 18	32.4	7.39	5.8	No tetany. Heavy meat diet.
		Feb. 21	Feb. 20	36.8	7.30	5.0	Tetany. Refused food. Given 3 mgm. uranium nitrate per kilo.
			Feb. 22	30.2	7.25	7.0	No tetany. In coma when bled. Died uranium-nitrate poisoning.

TABLE I—*Continued*

Dog	Operated	Tetany	Bled	CO_2 Cap. Vols. per Cent	pH	Ca Mgm. per 100 Cc.	Remarks
8.	Feb. 20		Feb. 20	41.2	7.40	10.8	
		Feb. 25	Feb. 25	34.2	7.36	5.9	Tetany. Spasm; unconscious 10 min. after bleeding. Injected 40 mgm. (5 per kilo) uranium nitrate.
			Feb. 27	28.7	7.22	6.2	Tetany disappeared in 24 hours.
			Mar. 2	22.3	7.24	6.0	Uranium-nitrate-poisoning symptoms present, however.
9.			Feb. 21	45.4	7.38	13.0	
	Feb. 23	Feb. 27	Feb. 27	45.5	7.37	7.0	Tetany. Injected 40 mgm. (5 per kilo) uranium nitrate.
			Mar. 2	21.1	7.23	7.5	Normal condition. Meat diet.
			Mar. 7	19.8	7.22	7.2	Symptoms of uranium poisoning; no tetany.
			Mar. 8				Animal died.
10.	Mar. 12		Mar. 12	45.4	7.35	12.4	
		Mar. 17	Mar. 17	42.4	7.34	7.4	Tetany. Injected 40 mgm. (5 per kilo) uranium nitrate. Normal after 36 hours. Eats meat; runs about.
			Mar. 19	37.3	7.33	8.2	No tetany symptoms. Heavy meat diet.
			Mar. 24	15.7	7.18	8.0	Uranium-nitrate-poisoning symptoms present. No tetany. Died.

Despite the fact that the dogs showed no gross manifestations of tetany and appeared normal, nevertheless the serum calcium remained low, in several cases as low as 5 mgm. of calcium per 100 cc. This was earlier observed in CO_2-treated animals.

The complete explanation why slight shifts in the acid-base equilibrium, with a change in reaction of the blood toward the acid side, should promptly relieve all tetany symptoms and bring about small increases in the serum calcium is not forthcoming in the present state of our knowledge of the pathogenesis of tetany. However, a tentative hypothesis, based upon facts which have recently come to light, is perhaps permissible.

It has been known for some years that blood serum contains more calcium than an ordinary aqueous solution of the same pH and phosphate content could maintain in solution. This fact has

been experimentally demonstrated by Holt, La Mer, and Chown (1925), who showed that when serum containing solid $Ca_3(PO_4)_2$ is shaken for some time, the concentration of both calcium and phosphate in solution decreases. This observation led Holt, La Mer, and Chown to suggest that blood serum is a supersaturated solution of calcium phosphate, and they advanced the hypothesis that serum contains a substance which delays the precipitation of calcium which is absorbed from the intestines.

Greenwald and Gross (1925*a*, *b*) observed that following parathyroidectomy there is a greatly diminished excretion of phosphorus in the urine, and furthermore that the retention of phosphorus is not, regularly at any rate, accompanied by an increase in the inorganic phosphate of the plasma. In later experiments the same investigators found that the decreased calcium content of the serum which invariably follows parathyroid removal is not due to increased excretion of calcium. In fact after gland removal, calcium excretion is much diminished, so that retention of calcium and phosphorus occurs. However, if an active extract of the parathyroid (Collip's) is injected, a marked increase in excretion of both calcium and phosphorus takes place. As a result of these observations, Greenwald (1925) advanced the hypothesis that in the absence of the parathyroid hormone, calcium and phosphorus are precipitated in the tissues in the form of calcium phosphate, thus accounting for the retained calcium and phosphorus and the simultaneous fall in the level of the serum calcium. According to Greenwald then, the parathyroid hormone, or some substance to the preparation of which the parathyroid hormone is essential, maintains calcium in solution in the plasma in excess of the quantity possible in its absence.

It becomes evident, therefore, that the ratio of calcium to phosphorus in the circulating fluids of the body of parathyroidectomized animals is highly important, and that anything tending to increase the elimination of phosphorus will make more calcium available. It seems probable that the relief from tetany symptoms and the rise in serum calcium which follows slight changes in the reaction of the blood toward the acid side, such as, for example, an intoxication by CO_2 or uranium-nitrate administration, is due to removal of phosphate. We have not demonstrated such elimination of phos-

phate in our experimental animals but are now engaged in this experiment.

While the increased excretion of phosphorus following development of acid intoxication remains to be proved for parathyroidectomized dogs, nevertheless such increased excretion has been demonstrated in cases of infantile tetany treated with acids and acid-forming salts such as ammonium chloride. Thus Scheer, György, and Anderson and Graham report that increased phosphate elimination occurs in such cases. It seems very likely that the relief of tetany which follows injections of uranium nitrate is due to increased phosphate excretion, which results from the shift in the reaction of the blood to the acid side, thereby rendering more calcium available owing to its greater solubility at the increased hydrogen-ion concentration.

During the course of the experiments an effort was made to determine the nature of the acid or acids responsible for the intoxication which follows injections of uranium nitrate.

Some years ago (1923) Denis reported that in severe uranium nephritis in animals, the inorganic sulphate of the blood is increased. In a later paper (1927), the same author reports that in dogs poisoned with uranium nitrate, the concentration of the inorganic sulphates and of the neutral sulphur fraction of the blood directly depended upon the volume of urine excreted. In animals with diuresis whose kidneys had been severely injured, there was frequently found a large decrease in the inorganic and total sulphates. As the volume of urine diminished to subnormal values, the concentration of inorganic sulphates and neutral sulphur rose; and in some cases attained value from 100 to 300 per cent above the normal concentration.

We tested two of our uranium-injected dogs for pH, CO_2 capacity, chlorides, phosphorus, and sulphates. The results of the tests are given in the combined protocols and tables.

URANIUM-NITRATE INJECTED DOGS

DOG A

White collie, male. Weight, 14 kilos. Uranium nitrate injected subcutaneously, 98 mgm. (7 mgm. per kilo of body weight). Bled

from heart 6 days later. Dog beginning to show symptoms of uranium-nitrate poisoning.

Bled 5 days later. Dog very weak; refused food for 24 hours; stiff when walking.

Died 3 hours after bleeding.

	pH	CO_2 Cap. Vols. per Cent	Cl Mgm. per 100 Cc.	P Mgm. per 100 Cc.	S Mgm. per 100 Cc
Normal	7.36	49.5	350	5	6.6
Six days	7.26	36.5	320	12	17.6
Terminal (11 days)	7.03	15.5	240	13.5	42.4

DOG B

Brown hound, male. Weight, 25 kilos. Uranium nitrate injected subcutaneously, 175 mgm. (7 mgm. per kilo of body weight). Bled from heart 6 days later. Dog beginning to show symptoms of uranium-nitrate poisoning.

Bled 4 days later. Dog very weak; refused food.

Died 2 days later.

	pH	CO_2 Cap. Vols. per Cent	Cl Mgm. per 100 Cc.	P Mgm. per 100 Cc.	S Mgm. per 100 Cc
Normal	7.40	51	340	5	3.9
Six days	7.11	31.5	340	14.25	14.9
Terminal (10 days)	7.09	25.1	270	15	37.6

It is evident from the data presented that marked retention of phosphorus and sulphate occurs following injections of uranium nitrate and that the blood chlorides fall. It seems probable that the acid intoxication which invariably accompanies uranium-nitrate poisoning is due in large part to retention of inorganic phosphorus and sulphate. The fall in chloride is obviously a compensatory reaction on the part of the organism in an effort to maintain the acid-base equilibrium in the face of retention of the other acids. The volume of urine excreted by our injected dogs diminished rapidly once uranium-poisoning symptoms developed.

SUMMARY AND CONCLUSIONS

1. Injections of uranium nitrate into parathyroidectomized dogs exhibiting marked tetany relieves all symptoms, and the animals return to an apparently normal condition within a few hours.

2. Dogs so treated remain normal for a week or 10 days before tetany recurs, despite the heavy meat diet given. Death results from uranium-nitrate poisoning.

3. Injected dogs, although free from tetany and apparently normal, have a low serum calcium. The serum calcium rises slightly some hours after injections, but the increase is seldom above 1.5–2 mgm. per 100 cc. of blood.

4. Uranium-nitrate injections induce a marked acid intoxication, as shown by lowered pH, CO_2 capacity, and CO_2 content.

5. The acidosis is probably due to retention of inorganic phosphate and sulphate. Blood chlorides tend to fall as phosphate and sulphate increase.

6. The relief from tetany symptoms which occur following uranium-nitrate treatment is apparently due to the shift in the reaction of the blood to the acid side, thereby rendering more calcium available owing to its greater solubility at the increased hydrogen ion concentration and to increased phosphate excretion.

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The Physiology of the Crustacean Heart. I. The Effect of Various Ions upon the Heart Rhythm of the Crayfish, *Cambarus clarkii*

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THE PHYSIOLOGY OF THE CRUSTACEAN HEART. I. THE EFFECT OF VARIOUS IONS UPON THE HEART RHYTHM OF THE CRAYFISH, CAMBARUS CLARKII

(Nine figures)

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INTRODUCTION

PROBLEMS concerning the relationship of various ions of the blood to the normal heart rhythm of crustaceans have received relatively slight consideration in comparison to the abundance of data available for certain other groups of cold-blooded animals. Moreover, the bulk of investigation upon the crustacean, with respect to the effect of ions, has dealt chiefly with the larger salt-water forms, notably the crabs and lobsters. In this connection attention is called to the following investigations: Rogers (1905), working upon the crab (*Brachynotus nudus*), found that Na, K, and Ca each have an important effect upon heart contraction. Hogben (1925), comparing the effect of ions upon the hearts of *Maia* and *Homarus*, found that there was a variation in the ionic requirement of the two species in that the concentration of K present in the perfusing solution determined whether or not the lack of Ca would produce systolic or diastolic arrest. He found, however, that an excess of K produced systolic arrest of the heart in both species. Hogben furthermore found that the hearts of both of these crustaceans were relatively insensitive to changes in H-ion concentration within the range of pH 5.5–7.2.

Comparatively few similar observations have been made on the fresh-water crayfish heart, although it provides favorable material for such an investigation. Hoffmann (1912), in the course of his investigation on action currents in the crayfish heart (*Astacus*), noted that an excess of either K or Ca produced little effect upon the normal heart rhythm. His chief concern in these ions lay only in finding a suitable perfusing solution for maintaining the normal heart pulsations.

An analysis of crayfish blood (*Astacus*) by Fredericq (1885), indicates a surprisingly low percentage of soluble salt content compared to the concentration determined for many marine crustaceans. In this connection it was found that the soluble salt content of *Astacus* was less than 1 per cent, while that of Palinurus and Homarus amounted to approximately 3 per cent. This suggests the probability that the ionic requirements for the maintenance of normal heart rhythm in the fresh-water crayfish might be somewhat different from that found true for salt-water species. It was with this idea in mind that experiments were planned to determine the effect of various ions, particularly Na, K, Ca, and Mg, upon the heart rhythm of the crayfish; and secondarily, to record any peculiarities or similarities due to ionic action as have been observed for other crustaceans.

It is with pleasure that I acknowledge my indebtedness to Dr. O. M. Helff for suggesting the problem and for his kindly interest and criticism throughout the course of the investigation.

MATERIALS AND METHODS

The crayfish employed for all experiments was *Cambarus clarkii*, a shallow-water species native to the swamps and fresh-water marshes of Louisiana. They are a very strong and active variety, ranging from 3 to 5 inches in length. The animals were shipped from Louisiana and upon arrival were placed in a large tank of running water for several days before being used for experimental purposes. The purpose of this precaution was to assure the animals' complete recovery from possible disturbances due to shipping, and also to acclimate them to any change in the environment.

The technique employed in obtaining graphic records of the effect of various ions on heart rhythm was briefly as follows: A piece of the carapace about 1 cm. square was removed from an area directly over the heart. This exposed the gills and the heavy body wall separating the carapace from the pericardium. A portion of the body wall at this point was then dissected away very carefully and the pericardium exposed. The pericardium was then partially removed, thus exposing the heart. The animal was now placed in a clamp which held it securely in a horizontal position and prevented

the movements of the appendages from interfering with the accuracy of the recording apparatus.

The recording apparatus consisted of the usual set-up, including a kymograph, heart lever, and signal magnet, the latter operated by means of an electrical clock timing apparatus. The comparative weakness of the heart pulsations of the crayfish necessitated the use of a heart lever which would reduce friction to a minimum. For this purpose a broom straw, balanced upon a very fine pivot, was employed. The heart was then attached to the lever by means of a very fine wire and steel hook, the latter being carefully inserted through the dorsoposterior region of the heart in order that the normal functioning of the ostia might not be impaired. The pulsations were then recorded upon a slowly moving drum.

It was found desirable to keep the heart perfused at a constant rate for all solutions employed. For this purpose a series of delivery siphons was arranged, one for each solution, whose rate of flow could be accurately regulated by means of screw clamps. The perfusing solutions were allowed to run into the pericardial sinus, from which point they were quickly perfused through the heart by way of the ostia. With this system of siphons it was possible to change the perfusing solutions very rapidly, while the temperature of the solutions could be checked continuously. The temperature of the perfusing solutions was maintained at $25 \pm 0.1^\circ$ C., which was generally the room temperature and also that of the aquarium water in which the stock animals were kept.

A series of perfusing solutions containing varying concentrations of NaCl, KCl, $CaCl_2$, and $MgCl_2$ were tested in an attempt to secure a perfusing bath capable of maintaining normal heart rhythm for a considerable length of time. In this connection Hoffmann's (1912) double-Ringers solution was tried, but it was found inadequate in maintaining normal heart rhythm for the species in question. It was finally found that a solution containing 1 per cent NaCl plus 0.028 per cent KCl plus 0.024 per cent $CaCl_2$ produced no noticeable effects on the normal rate, amplitude, or tone of the heart pulsations, even when applied over a period of several hours. This solution was employed as a control bath and as a basis for observing the effect of solutions containing different ionic concentrations. For con-

venience, the foregoing perfusing solution will be spoken of as the "normal perfusing solution" throughout the present paper and will be designated by *N* on all graphic records. The normal perfusing solution and all other ionic solutions were made with double-distilled water.

The experiments consisted in first perfusing the intact heart for several minutes with the normal perfusing solution. This removed most of the blood, not only from within the heart, but also from the surrounding pericardial sinus. After the rhythm of the heart had become normal, the normal perfusing solution was then discontinued, the ionic solution to be tested immediately applied at the same rate, and changes in heart rhythm recorded graphically.

In order to determine whether the various solutions employed actually entered and perfused the heart through the ostia, the following simple test was applied: A small quantity of powdered carmine was added to the perfusing solution being applied to the heart. By means of a hand lens, it was quite evident that the carmine granules were being sucked into the heart through the ostia, thus proving that the solutions applied to the surface of the heart are quickly perfused through that structure.

RESULTS

The effect of the Na ion.—The heart of the crayfish was found to beat normally for several hours when perfused with a solution containing 1 per cent NaCl plus 0.028 per cent KCl plus 0.024 per cent $CaCl_2$, but upon removal of the KCl and $CaCl_2$, a rapid decrease occurs in the diastolic tone, with an increased amplitude of the beats (Fig. 1). There is always a slight acceleration in the rate at first, but after 10 or 15 minutes the beats become less regular and the heart shows signs of typical sodium fatigue, eventually stopping in diastolic arrest. The arrest is generally brought about in the intact heart in less than 30 minutes. Upon doubling the NaCl in the normal perfusing solution, a slight decrease in diastolic tone follows, and also the characteristic increase in amplitude (Fig. 2). The rate is somewhat greater as compared with the results obtained when perfusing with a pure NaCl solution. Upon application of the normal perfusing solution, the normal rate and amplitude are first restored (Fig. 2); and later on, the normal tone. Upon the application of a

solution containing 0.028 per cent KCl plus 0.024 per cent plus $CaCl_2$ an immediate decrease in tone occurs, followed shortly by a sharp and continued increase in tone (Fig. 3). With continued perfusing, the rhythm is greatly depressed, the amplitude falling markedly until the heart goes into diastolic arrest. Subsequent application of a

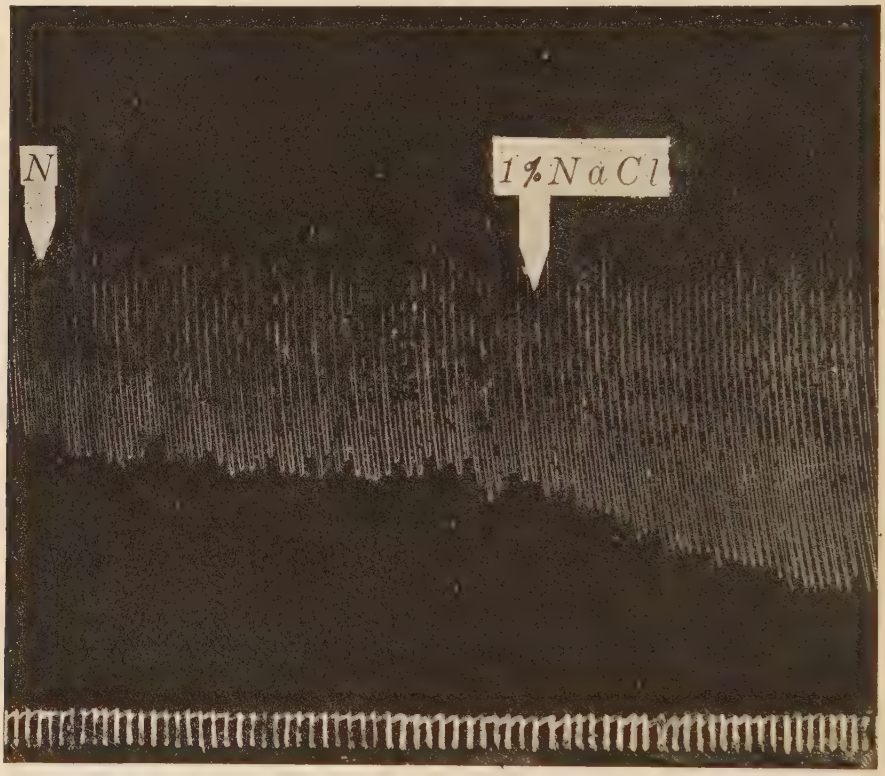

FIG. 1

normal perfusing solution results, in time, in complete recovery of the normal rhythm.

The effect of K and Ca ions.—Upon applying a 0.024 per cent $CaCl_2$ solution to the heart, a sharp increase in tone immediately follows, coupled with an irregularity in the rhythm (Fig. 4). The beats are not all of the same amplitude, and there is a decrease in the rate. If the $CaCl_2$ is increased in the normal perfusing solution, the

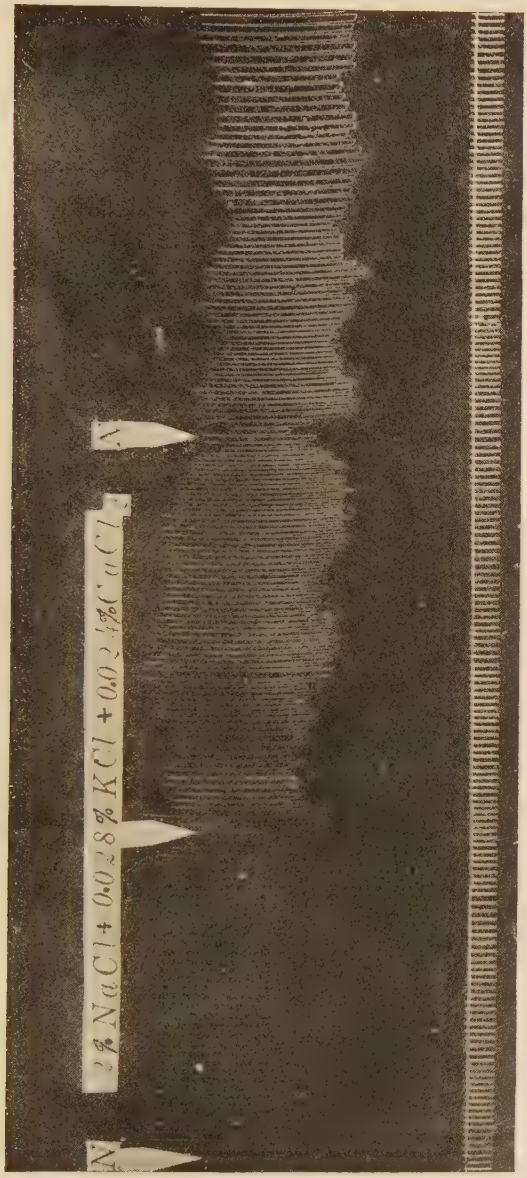

FIG. 2

rate is greatly decreased; but only slight changes in the tone and amplitude of the beats can be noted at first. Figure 5, for example, represents the depressor action on a heart perfused with a solution containing 1 per cent NaCl plus 0.028 per cent KCl plus 0.1 per cent $CaCl_2$, and in which the $CaCl_2$ concentration is approximately four times greater than in the normal perfusing solution. If the heart is perfused with a solution lacking in Ca, it will beat normally for some

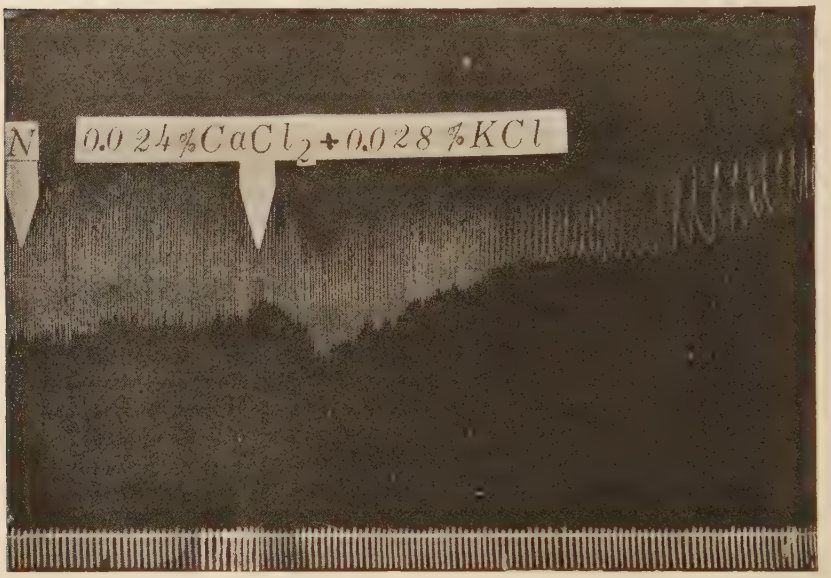

FIG. 3

time. Some hearts will continue beating for as long as $2\frac{1}{2}$ hours when perfused with a solution containing 1 per cent NaCl plus 0.028 per cent KCl, with but very slight variation in the normal rhythm. At the end of this time, short periods of systolic arrest occur, the heart finally stopping in systole. Subsequent perfusing with a normal perfusing solution for 10 minutes invariably failed to initiate pulsations.

The effect in absence of KCl is somewhat different. The use of a solution from which KCl is absent, results in complete stoppage of the heart in less than $\frac{1}{2}$ hour. Figure 6A is a record of a heart perfused with a normal perfusing solution, while Figure 6B represents the type of pulsations obtained at the close of a 25-minute applica-

tion of a solution containing 1 per cent NaCl plus 0.024 per cent $CaCl_2$. The heart, it will be noted, gradually increases in tone; while the relaxation period of each beat is greatly prolonged, resulting finally in systolic arrest. Upon the subsequent application of the normal perfusing solution, the normal rhythm is gradually restored.

A pure 0.028 per cent solution of KCl was found to have a depressor action on both the amplitude and rate, although the tone

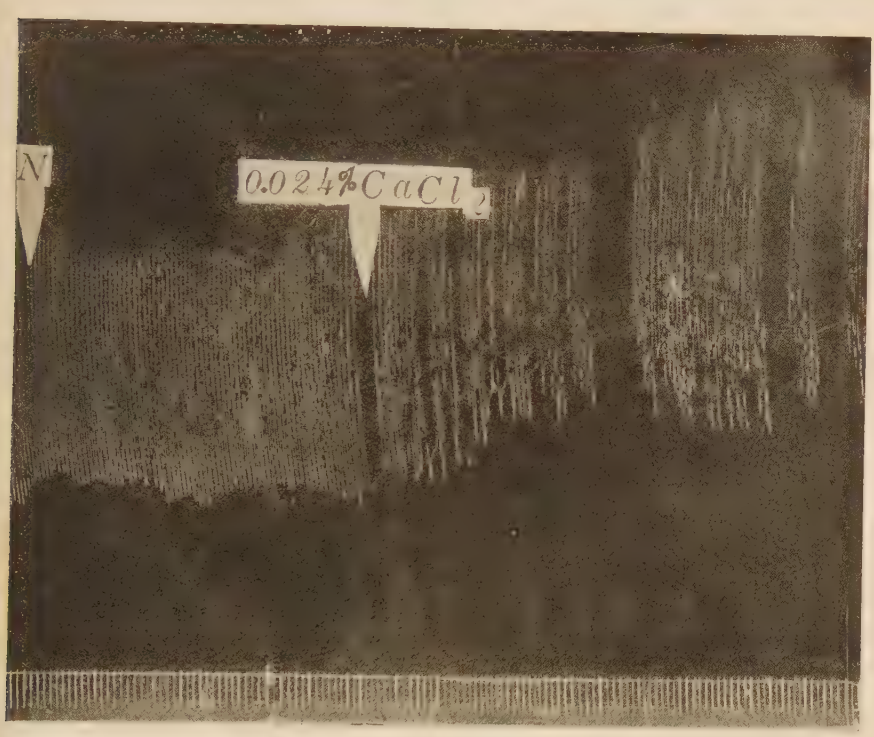

FIG. 4

appeared to be slightly increased. Complete relaxation of the individual beats is slightly prolonged with continued perfusion, resulting finally in systolic arrest. If the concentration of KCl is increased slightly in the normal perfusing solution, no noticeable change in rhythm can be detected. If, however, it is increased to 0.1 per cent, there is a characteristic decrease in the rate and amplitude of the beats coupled with a slight increase in tone. In addition, abnormal

rhythm, composed of several small beats superimposed upon each large beat, is obtained (Fig. 7).

FIG. 5

The effect of the Mg ion.—The heart of the crayfish beats normally in the absence of $MgCl_2$, while a slight amount in the normal perfusing solution produces no noticeable effects. A normal perfusing solution for example, to which has been added 0.02 per cent $MgCl_2$, produces no specific effect on the normal heart rhythm; and the heart will continue beating, on the average, for some length of time. If the $MgCl_2$ is increased, however, to 0.1 per cent, the rate is considerably decreased, although the tone and amplitude are but slightly changed.

The effect of the hydrogen ion.—The normal perfusing solution and the ionic solutions as used in determining the effect of various ions on heart rhythm in the present work had a pH of 7.0±.01. It seemed desirable to determine whether or not slight changes in the pH of the solutions employed may have had an effect upon the heart rhythm. To determine this, normal perfusing solutions were em-

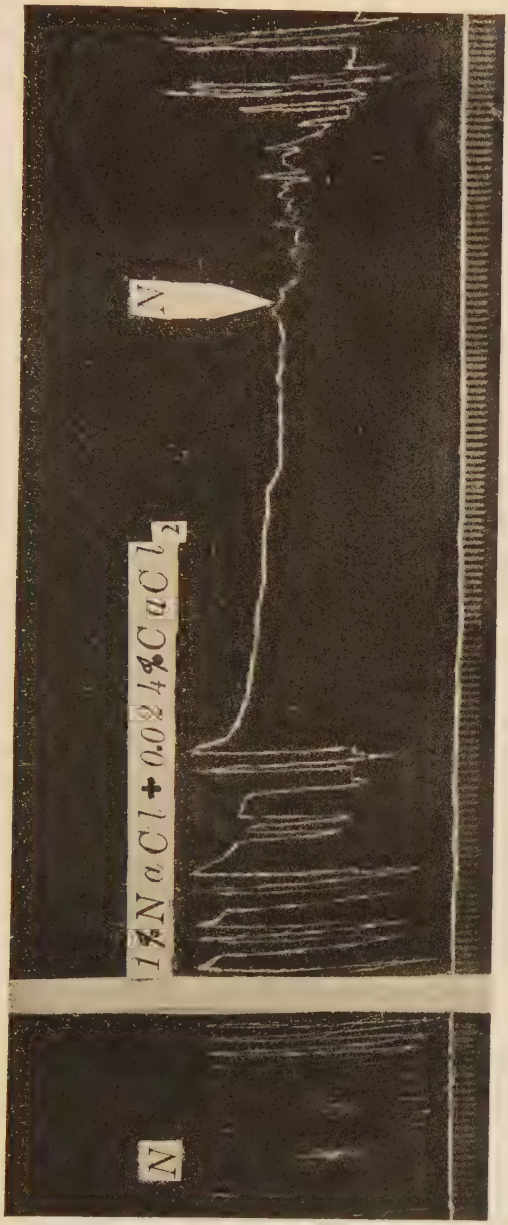

FIG. 6B

FIG. 6A

ployed whose pH had been lowered by means of CO_2 and HCl. It was found that the pH of the normal perfusion solution could be dropped suddenly from 7.0 to 5.5 without any appreciable change in the rhythm; but if dropped below 5.5, an increase in tone resulted (Fig. 8). It was also found that if the pH was dropped to 5.0, or any point below, the heart was immediately thrown into systolic arrest (Fig. 9). An interesting phenomenon was also noted when the pH of the perfusing solution was gradually lowered. The heart rhythm under these conditions remained normal when perfused down as low as pH 4.0, but was finally and gradually thrown into peculiar tone waves similar to those noted in a dying heart. It would appear, therefore, that the crayfish heart is able to adapt itself to relatively high H-ion concentrations, providing the pH is lowered gradually.

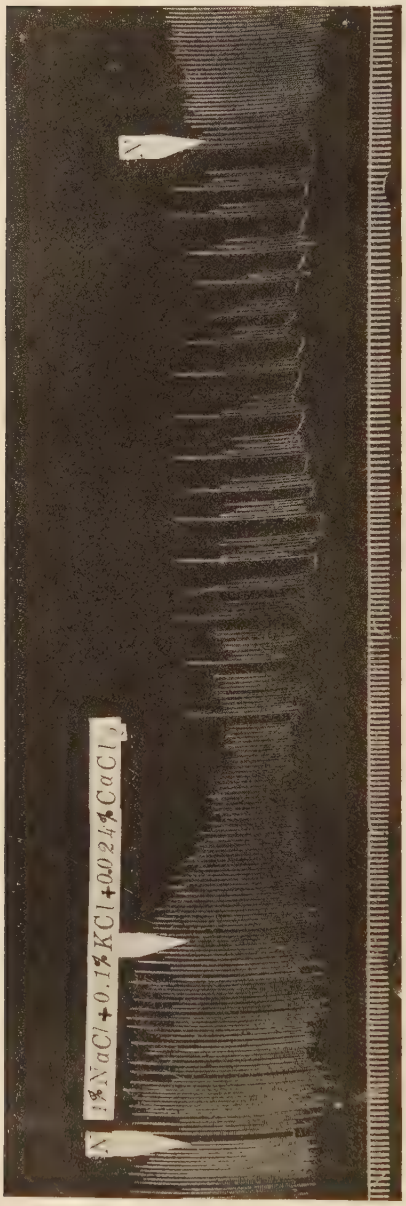

FIG. 7

By means of NaOH, the pH of the perfusing solution could be increased without changing the Na content greatly. It was found that such solutions having a pH up to at least 9.0 had little or no effect upon the normal heart rhythm.

DISCUSSION

A question might well be raised regarding the ionic concentrations of the perfusing solutions employed, as compared with the various known ionic concentrations of the crayfish blood. An analysis by Dohrn (1866) gives the following approximate percentage composition of *Astacus* blood: NaCl, 0.81 per cent; KCl, 0.28 per cent: $CaCl_2$, 0.47 per cent; and $MgCl_2$, 0.09 per cent. Griffith (1892), work-

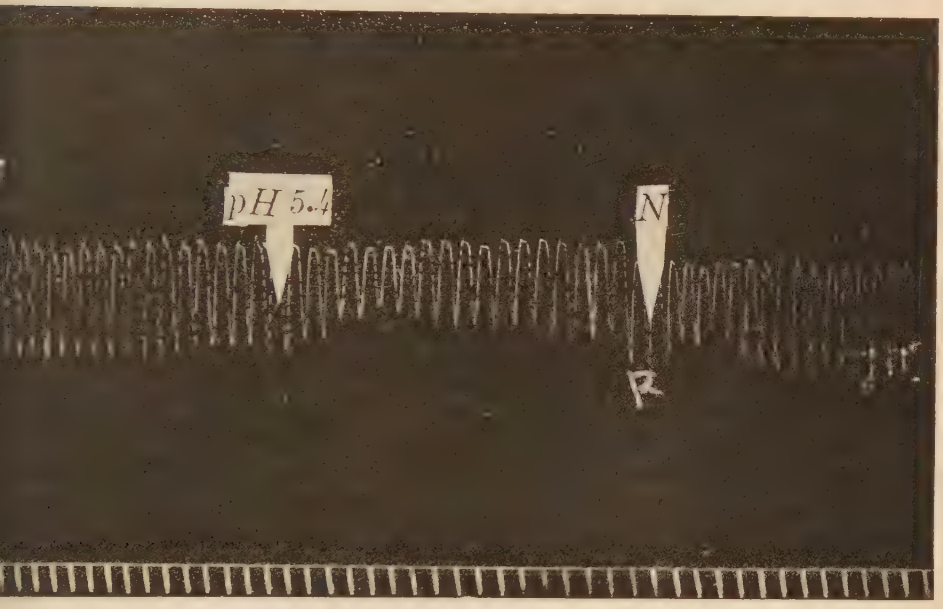

FIG. 8

ing on the same species, quotes somewhat lower concentrations of K and Ca, although the Na content was found to be approximately the same. The ionic concentrations of the perfusing solutions employed in the present work (with the exception of the Na) were much lower than those determined for *Astacus* blood. It was found that a perfusing solution, identical in ionic concentrations with those of *Astacus* blood, would not maintain the normal heart rhythm of *Cambarus clarkii*. However, if the concentration of NaCl was increased to 1 per cent and the KCl and $CaCl_2$ decreased to 0.028 per cent and 0.024 per cent, respectively, a solution was obtained which would maintain normal heart rhythm for a period of several hours.

A normal perfusing solution containing less than 1 per cent NaCl, operated to decrease the normal rate of the beats. The foregoing results, therefore, tend to indicate that the ionic content of the blood of *Cambarus clarkii* is somewhat different from that as determined by Dohrn for *Astacus*. Furthermore, the strengths of the ionic con-

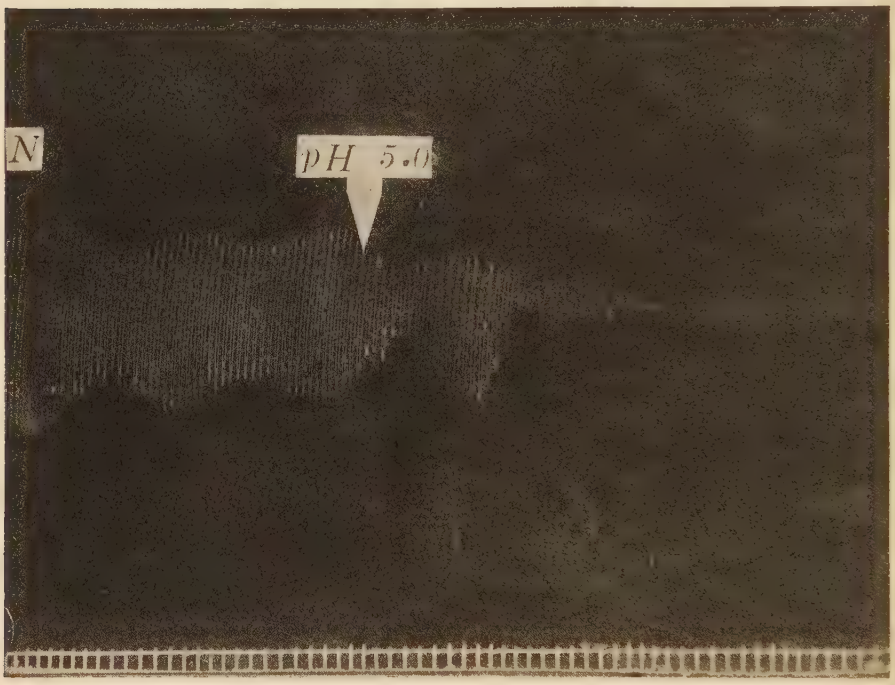

FIG. 9

centrations employed were such that the results obtained upon perfusion could not be attributed to toxic effects.

There has been considerable difference of opinion as to the rôle performed by Na, K, and Ca ions in the maintenance of heart tonicity in various animals. Ringer (1887), for example, working with the frog, noted that K and Ca ions displayed antagonistic properties in this regard. He found that an excess of the former ion decreased the tone, while the latter tended to increase it. Howell (1901), working with the terrapin, states that the antagonism is principally between the Na and Ca and that K exercises but a slight

influence. The results upon the intact heart of the crayfish show quite clearly that the Na and Ca ions are undoubtedly concerned in maintaining the tone. Furthermore, there are indications that K also has an important influence upon the tone. It has been shown that a heart perfused with 1 per cent NaCl invariably loses tone; but if perfused with one containing 1 per cent NaCl plus 0.028 per cent KCl, this pronounced loss in tone does not occur. It appears, therefore, that K, likewise, may have an antagonistic effect toward the Na ion.

It is also a question as to which of the three ions is most influential in the maintenance of the normal tone. Data have been presented which indicate that all exert some influence. The Ca and Na ions undoubtedly exhibit marked antagonistic effects, the former increasing and the latter decreasing the tone. The influence of the K ion upon the tone is somewhat problematic. It is undoubtedly not antagonistic to Ca in that a perfusing solution containing approximately equal ionic concentrations of the two causes a sharp increase in heart tone. It appears likely, therefore, that K and Ca exhibit similar properties in this respect and are each capable, to a greater or less degree, of antagonizing the effect of the Na ion. The difference in ionic concentration of the $CaCl_2$ and KCl necessary to completely antagonize the effect of a 1 per cent NaCl solution on the tone would indicate that the Ca ion exerts a stronger antagonistic influence than does the K ion.

That the Na ion exerts a distinctly opposite effect upon the rate and amplitude of the crayfish heart beat to that of the K or Ca ion appears quite evident. In every case (whether in a pure solution or in an excess in the normal perfusing solution) the Na was found to increase the rate and amplitude of the beats. This increase in rate and amplitude was probably due to the well-known irritating effect of the Na ion. Carlson (1909) noted that NaCl caused excitation of *Limulus* heart muscle in that the amplitude was increased. Hogben (1925), working upon the heart of *Maia*, however, observed that a pure solution of NaCl produced an almost immediate increase in tone, resulting shortly in systolic arrest. The results obtained by the latter worker, it will be noted, are not typical of the usual NaCl effect upon heart muscle. The results obtained for K and

Ca ions upon the crayfish heart, as regards rate and amplitude, are characteristic of the effects of these ions on heart muscle in general, in that the rate and amplitude are depressed.

The possible influence of the Mg ion on normal heart rhythm is interesting to consider at this time. Fredericq (1913), for example, noted that Mg was not essential in maintaining the heart rhythm of the octopus. Mines (1911), on the other hand, found Mg was very essential for the normal heart rhythm in *Pecton*. Clark (1927) states that, in general, the presence of Mg is not essential for the normal heart action of non-marine forms. There appears to be, therefore, considerable variation in the response of different invertebrate hearts to the Mg ion. In this respect, the crayfish heart was found to beat normally in the total absence of Mg. Moreover, slight traces in the normal perfusing solution had no marked effect. The results obtained for the crayfish, therefore, indicate that the Mg ion is not essential for the maintenance of normal heart rhythm.

An interesting condition exists in the crayfish heart in that it is quite insensitive, within certain limits, to changes in H-ion concentration. Normal heart rhythm, it will be recalled, was obtained when perfusing with solutions having a H-ion concentration as low as pH 5.5. A comparatively high pH (up to 9.0) likewise failed to evoke any irregularities in the normal rhythm. It will also be recalled in this connection that when the pH was lowered gradually (0.5 of a pH at a time), the heart could be made to respond normally under a much lower pH than if the drop in the pH had been made rapidly. In fact, normal rhythm was obtained by this method when the pH of the perfusing solution had been reduced as low as 4.0. Hogben (1925), in the course of his investigations upon the hearts of *Homarus* and *Maia*, likewise found that the hearts of these species responded normally down to a pH of 5.6. It would appear, therefore, that the crustacean heart may be relatively unaffected during rather extreme changes in H-ion concentrations. Whether this is an adaptation to the normal fluctuating H-ion concentration of crustacean blood or whether it only indicates an independence of normal heart rhythm so a relatively constant H-ion concentration of the blood, is an interesting question. It would seem profitable, in this connection, that a careful study be made of the H-ion characteristics of crustacean blood during consecutive periods of time.

SUMMARY AND CONCLUSIONS

1. A method was devised whereby the pulsations of the intact crayfish heart could be recorded and studied, graphically, while being perfused with various ionic solutions.

2. Various concentrations of NaCl, KCl, and $CaCl_2$ were tested in the perfusing bath in an attempt to find a solution which would maintain the normal heart rhythm. It was finally found that a solution containing 1 per cent NaCl plus 0.028 per cent KCl plus 0.024 per cent $CaCl_2$ fulfilled this purpose, the heart beating normally for several hours when exposed and perfused at a temperature of $25 \pm 0.1^\circ$ C.

3. In an attempt to determine the effect of the Na ion upon the heart rhythm, three perfusing solutions were employed: One consisting of 1 per cent NaCl; another of 2 per cent NaCl plus 0.028 per cent KCl plus 0.024 per cent $CaCl_2$; and the third of 0.028 per cent KCl plus 0.024 per cent $CaCl_2$. Both solutions containing Na increased the normal rate and amplitude although decreasing the tone. The third, in which Na was absent, decreased the normal rate and amplitude but increased the tone markedly. It is concluded that the Na ion is undoubtedly concerned in regulating the tone of the crayfish heart.

4. Similar experiments were performed with solutions of KCl and $CaCl_2$. A pure solution of $CaCl_2$ produces a sharp increase in the tone and a slight decrease in the rate. Increasing the $CaCl_2$ in the normal perfusing solution affected the rate only, the latter being decreased slightly. A pure solution of 0.028 per cent KCl invariably depressed the rate and amplitude but had slight, if any effect, upon the tone. The removal of K from the control perfusing solution resulted in systolic arrest of the heart in less than 30 minutes. Normal rhythm could subsequently be restored upon the replacement of KCl in the perfusing solution. In general, therefore, it may be said that K and Ca ions depress the rate and increase the tone of the crayfish heart, the Ca ion having an especially pronounced affect upon the tone.

5. The addition of small amounts of $MgCl_2$ to the normal perfusing solution produced no change in normal heart rhythm. When a large excess was added, however, a marked decrease in the rate was recorded. The presence of Mg in the perfusing solution does not

appear, therefore, to be essential for the maintenance of normal heart rhythm. Furthermore, it does not seem probable from the results obtained, that concentrations of $MgCl_2$, as determined by analysis of crayfish blood, could have any marked effect on the maintenance of normal heart rhythm.

6. Normal perfusing solutions in which the H-ion concentrations had been altered by means of HCl, CO_2, and NaOH showed no marked effect upon normal heart rhythm within a range of pH 5.5–9.0. Decreasing the pH below 5.5 results in a marked increase in tone. It is concluded that the crayfish heart is capable of maintaining its normal rhythm under a considerable range of H-ion concentration and is not, therefore, peculiarly adjusted to function normally only when under the influence of a constant blood pH.

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A COMPARATIVE STUDY OF THE ACID-BASE EQUILIBRIUM OF BILATERALLY NEPHRECTOMIZED AND ADRENALECTOMIZED DOGS

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For several years the writer (1927) and his students have been studying the effect of bilateral adrenal extirpation in cats and dogs, and reached the conclusion that one of the train of causes resulting in death from adrenal ablation is acid intoxication. As a result of our experiments the hypothesis was advanced that the adrenal cortex secretes a hormone which in some manner assists in maintaining the normal functioning of the kidney. We were interested in the fact that the type of acidosis which appears during adrenal insufficiency is similar to that occurring in uremia and the experimental nephritis produced by nephrotoxic substances such as uranium nitrate. As a further means of testing the idea whether or not the kidney is involved in adrenal insufficiency, the writer undertook to make a careful comparison of the symptoms and blood findings occurring in adrenal insufficiency and those which follow kidney extirpation. For details regarding adrenalectomy and the blood changes following the onset of adrenal symptoms reference is made to the previous publications from this laboratory on the subject. In the present paper only data pertaining to renal insufficiency will be set down in detail.

Clinicians and pathological chemists have been aware for some years that the terminal stages of certain types of nephritis are complicated by a well-marked acid intoxication. In fact uremic symptoms are practically if not always accompanied by chemical changes in the blood indicative of acidosis. The acidosis is very probably not the cause of uremia but is associated with it. The blood of uremic individuals with acid intoxication shows lowered CO_2 capacity, CO_2 content and pH, and increase in phosphate and sulphate ions and non-protein nitrogen. The change in the acid-base equilibrium may be so great as to be the actual cause of death in some cases, according to Chase and Myers (1920).

The assumption has been that the acidosis of renal insufficiency is primarily due to retention of the acids formed in the course of normal metabolism due to inability of the injured kidney to excrete them.

However, it is not at all clear that total cessation of kidney functioning

results in acid intoxication. Thus Ptaszek (1927) recently reported that he was unable to obtain very marked symptoms of acid intoxication in dogs surviving 2 to 5 days after double nephrectomy. There was a small diminution in alkali reserve but the slight decrease was in marked contrast to the fall observed in cases of human uremia. He expresses the opinion that when the kidneys are extirpated some extra renal mechanism maintains the alkali reserve approximately normal, whereas in uremia the extra-renal mechanism breaks down along with the kidney.

Andrews (1927) reports several cases of dogs surviving 4 to 10 days following total nephrectomy. The animals did not show evidences of acid intoxication even in the terminal stages. They were active, played about and had good appetites.

While the writer is not directly concerned in the present pages with the problem of uremia, nevertheless some of the data suggest that the primary cause of uremia, is probably extra-renal in origin and that possibly the adrenal glands, and especially that portion known as the cortex, may be involved.

Bilateral nephrectomy. Large, well-nourished dogs, averaging in weight between 14 to 20 kilos, were employed as experimental animals. It has been our experience that large animals survive bilateral nephrectomy longer than the smaller dogs. They were kept in the laboratory for several days before using in order to accustom them to their new surroundings. They were fed cooked meat scraps, bread and occasionally a half pint of milk. The right kidney was removed first through an incision nearly parallel with the last rib. The operation is not severe and can be accomplished with practically no bleeding, the animal rapidly recovers and is normal, after 5 to 10 days the left kidney was extirpated in a similar fashion. Such operated dogs behave in normal fashion for two and sometimes three days. They are lively, eat and drink and walk about after the operation.

Several days following unilateral nephrectomy the animals were bled from the heart and from 60 to 70 cc. of arterial blood taken for chemical examination. Determinations were made of the CO_2 capacity, CO_2 content, pH, inorganic sulphate and phosphate, sugar, chlorides and urea. The methods employed were as follows: CO_2 capacity and content, Van Slyke (1927), pH, Myers, Schmitz and Booher (1923), using Myers and Muntwyler's correction for dog plasma (1927), sulphate, Denis (1927); phosphate, Benedict and Theis (1924); sugar, Folin (1922); chlorides, Whitehorn (1920); urea, Myers, (1924).

Following removal of the remaining kidney the animals were again bled when marked symptoms developed, but in all cases the determinations were made, while the animal was in good condition and before it became moribund. In several animals three determinations were made, the final

blood being taken just before the pre-mortal coma set in. It was found impracticable to bleed the animals frequently after the onset of symptoms since a considerable quantity of blood was required for the analysis. Too frequent bleeding weakens the animals and greatly shortend the life span of the nephrectomized dogs. Generally bilaterally nephrectomized dogs are normal for about 40 to 50 hours after the second operation, and occasionally are normal for three days. They eat, walk about and seem in excellent condition. The first symptoms are malaise and loss of appetite. The dogs sniff at proffered food and may eat a bite or two and then turn away. They appear listless, drink considerable water and lie quiet for hours at a time, but when aroused, will walk about in normal fashion. Vomiting is generally a prominent symptom, especially on the third day after the operation. The dogs vomit large quantities of clear fluid and mucus. If fed milk by the stomach tube it is usually vomited within a short time. One of the animals did not vomit at any time during the five days it survived double nephrectomy, and another animal vomited but twice during a four day survival period. In general however, vomiting is a prominent symptom. As symptoms progress, the animal shows marked weakness of the hind legs and sways from side to side when walking. In the terminal stage the animal passes into coma and dies. Fifteen dogs were used in the work. Two animals slipped ligatures within forty hours after the second operation and died of hemorrhages. Three dogs died during the night before the second blood was obtained for analysis, one had survived the second operation 74 hours, one 81 hours and the third 93 hours, and one animal developed pneumonia after the second operation and was not used.

In table 1 are presented the data from eight of the double nephrectomized animals.

An analysis of the data presented in table 1 reveals some interesting facts.

It seems quite evident that acid intoxication is not only not a prominent feature of the syndrome following bilateral nephrectomy but is entirely absent from most cases, and only doubtfully present in a few individuals.

It is somewhat surprising to find that total loss of renal function is not associated with acidosis. In uremia, acidosis is almost invariably present and in some cases, according to Myers and Chase, is of sufficient magnitude as to probably be the actual cause of death.

The assumption has commonly been made that the acidosis observed in uremia and that resulting from nephrotoxic substances such as uranium nitrate, is probably due to renal insufficiency with resultant piling up in the blood of acid metabolites normally excreted by the kidney. Such appears not to be the case, however, since total loss of kidney function, e.g., bilateral extirpation does not produce such intoxication.

TABLE 1

Blood findings after nephrectomy

DOG	DATE	CO_2 CONTENT	CO_2 CAPACITY	pH	S.	P.	UREA	SUGAR	Cl	REMARKS
		vol. per cent	*vol. per cent*		*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	
I	December 2	43.2	50.6	7.39	2.3	6.0	16	100		Normal
	December 6	40.4	46.7	7.34	6.9	8.5	55	100		18 hours post-operative
	December 8	40.3	47.7	7.32	28.2	10.0	84	103		66 hours post-operative
II	December 3	38.9	45.5	7.41	4.6	5.0	16	120		Normal
	December 13	31.3	39.4	7.33	19.7	10.0	90	145		70 hours post-operative
III	December 13	37.1	44.8	7.43	5.3	4.5	12	97		Normal
	December 18	32.3	43.7	7.36	30.6	12.5	83	110		72 hours post-operative
IV	December 13	40.3	47.6	7.37	5.1	5.5	20	117		Normal
	December 18	33.4	45.1	7.33	25.7	10.0	91	125		72 hours post-operative
	1928									
V	January 3	39.9	48.1	7.40	1.8	4.5	15	87	325	Normal
	January 6	43.9	47.5	7.38						72 hours post-operative
	January 8	36.5	40.3	7.35	31.2	15.0	100	105	220	105 hours post-operative
VI	January 9	41.6	49.0	7.38	2.7	5.0	15	91	320	Normal
	January 14	42.8	48.5	7.38	30.7	12.5	85	133	240	72 hours post-operative
VII	January 9	40.3	47.8	7.37	2.5	5.5	17	105	300	Normal
	January 17	35.5	42.8	7.38	23.6	13.2	100	118	240	98 hours post-operative
	January 18	34.6	41.0	7.38	37.7	16.2	112	122	221	120 hours post-operative
VIII	March 30	40.7	50.6	7.37	2.9	5.0	14	87	350	Normal
	March 31									
	April 1	39.2	47.6	7.35	27.7	12.0	95	87	280	72 hours post-operative
	April 2	32.1	42.5	7.35						88 hours post-operative

A second point of interest as shown by the data in table 1 is the fall in blood chloride which occurs following total nephrectomy. Chloride was not determined in our earlier cases. But when it became evident that acid intoxication was not present and that the animals vomited considerably, chlorides were determined.

In dog 5 the normal chloride concentration of the blood was 325 mgm. per 100 cc. Following bilateral nephrectomy (105 hours) when the animal presented marked symptoms the chlorides had fallen to 220 mgm. per 100 cc. Without exception blood chlorides diminished in the nephrectomized individuals.

Atchley and Benedict (1927) observed diminution in the chlorides following bilateral ureter ligation of dogs. They studied the chlorides lost in the vomitus and excreted in the urine and feces, but were unable to account for the loss on the basis of vomiting or excessive excretion. Study of chloride concentration of various tissues such as muscle and brain likewise failed to show deviations from the normal. They were unable to account for the fall in blood chlorides on the basis of their examinations.

Blood sulphate, phosphate, and urea increased markedly in the operated animals. Some of the values for sulphate were very high. Atchley and Benedict (1927) observed in their animals, that as the blood sulphate and phosphate rose, chlorides fell, and they state that the fall in chlorides offset the rise in sulphate and phosphate in so far as the effect upon the acid-base equilibrium was concerned.

In the present experiments one dog which survived bilateral nephrectomy 88 hours did not vomit during this interval—nevertheless the blood chlorides fell from the normal of 350 mgm. per 100 cc. to 280 mgm. seventy hours after the second operation. It seems probable, therefore, that the diminution in blood chloride can not be due to loss of chloride in vomitus.

Increase in blood sugar was observed after nephrectomy in all cases except animal 8. Animal 1 showed practically no change from normal. The remaining seven dogs showed slight increases up to the time of death. The increase in sugar was not high but sufficient to be readily detected. It appears, therefore, that a slight hyperglycemia is characteristic of nephrectomized dogs despite the fact that such animals take little or no food after symptoms develop.

Summing up the chief blood findings in bilaterally nephrectomized dogs it may be stated: 1. An uncompensated acid intoxication is not a symptom of renal insufficiency induced by kidney ablation. 2. Marked retention of sulphate, phosphate and urea occurs. 3. Blood sugar increases somewhat and remains above normal in the terminal stages. 4. Blood chlorides diminish, the decrease probably not being due to chloride lost in the vomitus since one of the dogs did not vomit but chlorides fell nevertheless.

Bilateral adrenalectomy. Detailed reports of chemical studies of

the blood of normal, unilaterally and bilaterally adrenalectomized dogs have been made elsewhere by the writer and his co-workers, and will not be repeated here except in so far as the results have a direct bearing upon the problem in hand.

It was observed that the chemical analysis of the blood of bilaterally adrenalectomized cats and dogs without symptoms showed no deviations from normal. However, when the first symptoms, such as anorexia and lassitude present themselves, a decrease in blood sugar is also present. In contrast to nephrectomized dogs whose blood sugar rises after the onset of symptoms, adrenalectomized dogs show marked lowering of the sugar of the blood when adrenal insufficiency symptoms are present. The sugar values may fluctuate up and down if considerable quantities of food such as milk are administered by stomach tube, but as the symptoms progress so also does the hypoglycemia. As the animal nears the terminal stage of adrenal insufficiency, i.e., coma, blood sugar values as low as 50 to 60 mgm. per 100 cc. are common. The hypoglycemia though marked, especially in the terminal stages, is not the cause of death, since injections of glucose although they raise the blood sugar to normal do not greatly prolong life.

Adrenal insufficiency symptoms in dogs and cats are invariably associated with acid intoxication; in fact, it is the writer's opinion that the symptoms of adrenal insufficiency are simply those of a slowly developing acid intoxication due to retention of non-volatile acids such as phosphate, sulphate and probably organic acid. Bilaterally adrenalectomized dogs may remain perfectly normal for three, four or six days following the second operation. They eat and play about in normal fashion. Chemical examination of the blood reveals nothing unusual. With the first signs of adrenal insufficiency such as total anorexia and lassitude, a marked decrease in blood sugar occurs and the CO_2 capacity falls without change in pH. This condition may last for a day or even two days, and as the symptoms progress the CO_2 capacity, CO_2 content and pH fall markedly. Near the terminal stages the CO_2 capacity may be as low as 18 vols. per cent, the CO_2 content 10 or 12 vols. per cent and the pH 7.10. The progress in symptoms appears to run parallel with the development of the acid intoxication.

Table 2 presents the blood findings in two typical cases. The data in this table should be compared with those in table 1 which shows the blood findings after nephrectomy. About fifty adrenalectomized dogs have been studied in this laboratory during the past year and the data given in table 2 are representative of the entire group.

In this laboratory chemical analysis of the blood is not made on animals which survive bilateral adrenal extirpation less than four days. Some of our dogs have survived 12, 10, 9, 8, 7 and 6 days. Such animals invariably

remain normal for several days following removal of the remaining gland. Many of our animals survive shorter intervals, 48, 60, 72, 80 and 91 hours. All such cases are carefully observed but detailed blood studies not made, since the survival period was considered too brief.

It is exceedingly difficult to make repeated blood determinations on adrenalectomized dogs when such large quantities of blood are necessary as in the present study, for repeated bleeding weakens the animal and

TABLE 2

Blood findings after adrenalectomy

Dog	Date of bilateral operation	CO_2 content	CO_2 capacity	pH	Ca	P	Sugar	Cl*	Remarks
	1927	*vol. per cent*	*vol. per cent*		*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	
38	June 8	41.3	49.2	7.39	2.2	4.8	102		Normal. Bilateral 24 hours. No symptoms
	June 12	32.1	37.3	7.34	5.4	7.1	70		Anorexia, lassitude, first symptoms
	June 14	24.3	29.1	7.28	8.3	8.2	72		Acidosis. Weakness. Marked symptoms
	June 15	18.4	23.4	7.21	10.4	10.7	62		Acidosis. Marked symptoms. Survived 7 days
44	October 7	40.6	48.2	7.40	1.9	5.1	107		Normal. Bilateral. No symptoms
	October 12	35.2	40.1	7.39	2.4	6.1	103		Some anorexia and lassitude
	October 14	29.1	34.2	7.33	5.7	7.6	74		Symptoms. Total anorexia and lassitude
	October 15	17.4	21.5	7.17	11.4	10.1	65		Marked symptoms. Acidosis. Weakness. Survival 8 days

* Chlorides not determined.

induces exacerbation of symptoms when they are present. It is rarely possible to obtain several sulphate and phosphate determinations on the same animal along with the other blood constituents. In only two of our animals has it been possible to do this. The sulphate determinations were usually omitted in the dogs when the other blood constituents were determined.

The data presented in table 2 show conclusively that a marked uncom-

pensated acid intoxication is characteristic of adrenal insufficiency in dogs and is apparently due to retention of phosphate and sulphate.

Chlorides were not determined in our dogs since the writer in earlier work (1927) on cats found that they do not change appreciably following adrenal removal. Lucas (1926) however, reports that in bilaterally adrenalectomized dogs, chlorides are decidedly diminished.

Urea and non protein nitrogen were not determined since it is well known that following adrenal removal in both cats and dogs these substances greatly increase (Marshall and Davis, 1917; Lucas, 1926; Swingle, 1927).

Discussion. In previous papers the writer has stated that the probable cause of death in adrenal insufficiency is a slowly advancing acid intoxication due to retention of the acid end products of normal metabolism. It was postulated on the basis of the blood analysis that the kidney fails to properly perform its acid eliminating functions in the absence of the hormone of the suprarenal cortex, and that the latter supplies something which is necessary for normal renal activity. Histological examination of the kidneys of adrenalectomized cats failed to reveal any gross pathological changes, but recently Hartmann (1927) and collaborators report lipoid nephrosis following adrenal extirpation in cats.

However this may be, it is evident from the present study of the comparative effects of total kidney removal and bilateral adrenal extirpation that the acid intoxication which is so prominent a feature of the adrenal insufficiency syndrome is not entirely dependent upon impaired kidney function since it does not appear in dogs deprived of both kidneys where failure of renal function is complete.

Phosphate and sulphate retention are even more marked in nephrectomized than in adrenalectomized animals, yet an approximately normal acid-base equilibrium is maintained.

The conditions reported for human uremics are similar to those observed following adrenalectomy in regard to the nature of the acid intoxication. In uremia, it is generally assumed that the acidosis is due to retention of phosphate and sulphate by a diseased kidney.

It seems to the writer that too much stress has been laid upon the kidney as the site of lesion responsible for the acid intoxication of both adrenal insufficiency and uremia. It is suggested here, that possibly one reason why bilaterally nephrectomized dogs maintain an approximately normal acid-base equilibrium up to the time of death, is that an extra-renal factor is responsible for the maintenance of the normal equilibrium and that the mechanism may consist of an active principle or hormone elaborated by the suprarenal cortex. In nephrectomized dogs the kidney function is eliminated but the suprarenal cortex remains intact and assists in maintaining normal equilibrium. In uremia, on the other hand, this extra-renal factor, i.e., suprarenal cortex, breaks down along with the kidney

function. In adrenalectomy the conditions would be essentially similar to those of uremia since loss of the suprarenal cortex would lead to acid intoxication, the kidney lesions being of secondary importance.

SUMMARY

1. Bilaterally nephrectomized dogs do not develop acid intoxication. In some cases a slight fall in alkali reserve may occur but the change is in striking contrast to the acidosis which follows adrenal removal.

2. Blood sugar increases slightly after total kidney removal but falls markedly after adrenal extirpation.

3. High figures for inorganic phosphate, sulfate and urea are found after nephrectomy and adrenalectomy.

4. Blood chlorides fall as sulfate and phosphate rise following nephrectomy.

5. The suggestion is made that the acid intoxication of adrenal insufficiency and probably also of uremia is extra renal in origin since it is not present in nephrectomized dogs with total suppression of kidney function.

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4207

Effect of Intravenous Injections of Alkali on Physiological Action of Curare.

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In an earlier paper[1] it was shown that a decrease in the pH of the blood, produced prior to the administration of lethal doses of strychnine, prevented violent tetanus and death. It was considered important to try the reciprocal experiment, that is, the effect of administering alkali to dogs that had previously received a lethal dose of curare.

Dogs used in this experiment received, intravenously, 5.7 mg. of curare per kilo of body weight. The drug used was the product of Burroughs, Wellcome & Co. Eight or 10 minutes after injection, when paralysis of the muscles, except the respiratory muscles, had appeared, a 5% solution of $NaHCO_3$ was injected intravenously. Complete recovery occurred within 15 minutes after approximately 3.0 gm. of $NaHCO_3$ had been introduced. The "curare effect" did not reappear.

At the onset of muscle paralysis the pH and CO_2 capacity begin to fall. The acidosis produced evidently augments the effect of curare but is not, in itself, sufficiently pronounced to produce coma. Introduction of $NaHCO_3$ not only corrects anoxemia but also possibly neutralizes the curare effect at the myo-neural junction.

All controls died of respiratory paralysis within 20 minutes after receiving curare.

The following table gives the data from one of our experimental animals:

[1] Wenner, W. F., and Blanchard, E. W., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 726.

TABLE I. Dog No. 5, weight 7.5 kg.

Time	Amt. Curare given	Amt. $NaHCO_3$ given	Blood pH	CO_2 Capacity Vol. %	Remarks.
10:40			7.39	42.0	
10:42	40.5 mg.				Injected intravenously.
10:50			7.27	40.2	Muscle paralysis. Dog still breathing.
10:51 to 10:53		2.5 gm.			Injected intravenously.
10:55					Reflexes returned.
11:15			7.46	47.6	Complete recovery.

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Histolytic Influence of Atrophying Gills During Metamorphosis: Special Reference to Resistance of Fore-limb Integument.

E. R. VAN DER JAGT. (Introduced by O. M. Helff.)

From the Zoological Laboratory, State University of Iowa.

Helff[1] has shown that the perforations in the opercular integument are the end result of an orderly process of cellular histolysis, due to the histolytic action of the atrophying gills. The present investigation was designed to determine the stage of gill atrophy at which this histolytic influence is most effective.

Homoplastic transplants of gills in various stages of atrophy were made to regions beneath the back skin of metamorphosing *Rana pipiens* tadpoles. The following constitute the main stages of gill atrophy investigated: (1) a stage showing the earliest macroscopic signs of atrophy; (2) a stage just prior to the release of the fore-limb; (3) a much later stage in which the integuments of the opercular region and the fore-limb had fused, following the release of the latter. Other atrophying gill transplants were made of stages intermediary to these, but the differences in the histolytic action were more clear cut when comparing the transplantation results of the 3 stages mentioned above.

Typical perforations were formed in the skin of the back directly in contact with the gill transplants. Histological sections of integument bounding such perforations showed that histolysis had taken place, identical to normal opercular integumentary histolysis. The stage of greatest histolytic influence of the atrophying gill was determined by the length of time necessary for the transplant to form a uniform sized perforation in the integument. This stage was found to be that just prior to the breaking through of the fore-limb. At this stage the gills are of a reddish hue, quite mushy in consistency, and capable of producing a perforation within 2 days.

The results indicate that the histolytic influence of the atrophying gills is cumulative. It is very weak during the early stages of gill atrophy, but increases proportionately with increasing atrophy and

[1] Helff, O. M., *J. Exp. Zool.*, 1926, xlv, 1.

reaches a maximum just prior to the release of the fore-limbs. Following the release of the fore-limbs the histolytic influence of the gills still persists, but gradually subsides as the gills undergo their final stages of atrophy.

During metamorphosis the fore-limbs differentiate very rapidly and occupy a large part of the opercular cavity. They were in contact with the atrophying gills which appear, however, to be ineffective in producing histolysis of the fore-limb integument. Operations were designed to throw light on this apparent resistance of fore-limb integument to histolysis.

In determining the possible specificity of fore-limb integument in this respect, autoplastic fore-limb integument transplantations were made to regions of the back. Homoplastic gill transplants, in the stage of greatest histolytic influence were then placed beneath the skin grafts. In this manner atrophying gill tissue was brought in contact with the *inner surface* of the fore-limb integument, a condition never attained during the normal development of fore-limb integument in the opercular cavity.

Perforations appeared in the skin grafts in all cases, being preceded by a definite process of thinning corresponding closely to the macroscopic thinning of normal opercular integument. The thinning was first noticeable in the integument directly over the slight bulge caused by the gill transplantation, and resulted in a loss of coloration, the area assuming a grayish and opalescent shade typical of histolysis. Histological sections later showed that the perforations were the result of a definite and typical cellular histolysis.

Homoplastic gill transplants in stages of greatest histolytic activity were next placed on the epidermal surface of fore-limb integument grafts, which had been previously transplanted to the back. Normal back skin was then grafted over both the gill and fore-limb integument grafts. After a typical perforation had appeared in the back skin graft, the 2 skin grafts were removed and sectioned. It was at once evident that the fore-limb integument showed only slight sign of histolysis as compared with the total disintegration of the back skin where the perforation had formed.

The above results indicate strongly that the failure of the integument of the fore-limbs to histolyze during metamorphosis when in contact with the atrophying gills, is due to the fact that the gills are in contact with the *epidermal surface* of the skin only. It must also be borne in mind that the integument of the fore-limb is undergoing rapid growth and development during metamorphosis and that the heightened metabolism of this tissue would also probably tend to counteract the histolyzing influence of the atrophying gills.

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4210

Effect of Changes in Ion Concentration of Blood upon Reflex Time.

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Previous studies[1, 2] on the effect of changes in acid-base equilibrium on the physiological action of strychnine and curare indicated that an increase in hydrogen or hydroxyl ions might affect neural or neuro-muscular factors and thus alter the rate of conduction of the nerve impulse.

Dogs were used as experimental animals. The patellar tendon reflex time of normal unanesthetized dogs varied from .00775 to .0094 seconds with an average of .0082 seconds. The pH of the blood of the normal dogs at the time the records were obtained was about 7.36 and the CO_2 capacity 45 volumes %. With a drop in pH and CO_2 capacity the reflex time was decreased to about .0043 seconds. An increase in pH and CO_2 capacity also brought about a decrease in reflex time. The average reflex time following alkali administration was .0057 seconds. One dog included here showed an insignificant increase of .0006 seconds in reflex conduction rate at pH 7.53 and CO_2 capacity 72.97 volumes %.

The records obtained show clearly that the reflex time of dogs is markedly reduced, following acid, and to a less degree, following alkali administration.

It is suggested that an increase in H ions probably blocks out the higher centers ordinarily involved in the arc. On the other hand OH ions probably aid in facilitating the conduction of the nerve impulse.

[1] Wenner, W. F., and Blanchard, E. W., Proc. Soc. Exp. Biol. and Med., 1928, xxv, 726.

[2] Wenner, W. F., and Blanchard, E. W., *Ibid.*, 1928, xxvi, (preceding abstract).

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4212

Metamorphosis of the Colorado Axolotl by Injection of Inorganic Iodine.

W. R. INGRAM. (Introduced by W. W. Swingle.)
From the Zoological Laboratory, State University of Iowa.

Experiments with the Colorado Axolotl, to be properly controlled, must be performed with hypophysectomized and thyroidectomized individuals, since this variety of axolotl metamorphoses spontaneously when removed from its native habitat. Individuals submitted to such treatment have been considered never to metamorphose except under the influence of a glandular replacement therapy or upon the administration of organic iodine compounds. Jensen,[1] Huxley and Hogben,[2] Huxley,[3] and Uhlenhuth,[4] in view of their experiments maintain that the transformation of urodeles cannot be accelerated by feeding inorganic iodine. However, Hirschler[5] was successful in bringing about the metamorphosis of one European Axolotl by injection of Lugol's solution. This type of axolotl never metamorphoses spontaneously. Blacher and Belkin[6] reported normal and hypophysectomized European axolotls brought to the adult condition by intraperitoneal implantations of small crystals of inorganic iodine.

The writer has conducted somewhat similar experiments with the Colorado Axolotl, and, following the subcutaneous implantation of powdered iodine crystals, hypophysectomized and thyroidectomized individuals rapidly passed through the transformation stages to the adult. The experimental animals were either kept long enough

[1] Jensen, C. O., *C. R. Soc. Biol.*, 1921, lxxv, 391.

[2] Huxley, J. S., and Hogben, L., *Proc. Roy. Soc.*, B. 1922, xciii, 36.

[3] Huxley, J. S., *Proc. Roy. Soc.*, B. 1925, xcviii, 113.

[4] Uhlenhuth, E., *Biol. Bull.*, 1922, xlii, 143; *ibid.*, 1923, xliv-xlv, 303.

[5] Hirschler, J., *Arch. Entw. Organ.*, 1922, 51, 482.

[6] Blacher, L. J., and Belkin, R. I., *Trans. Lab. Exp. Biol, of Zoopark of Moscow*, 1927, iii, 97.

after operation to preclude the possibility of small bits of glandular tissue remaining, or were killed following metamorphosis and the gland sites carefully examined. The controls all metamorphosed spontaneously within a month after arrival at the laboratory.

These results confirm the observations of Blacher and Belkin upon the European Axolotl. Although most investigators have considered inorganic iodine to have negligible influence upon the metamorphosis of urodeles, it now appears that large amounts of this element administered subcutaneously or intraperitoneally do markedly accelerate differentiation in neotenic forms.

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STUDIES ON SEX DIFFERENTIATION AND SEX DETERMINATION IN AMPHIBIANS

I. DEVELOPMENT AND SEXUAL DIFFERENTIATION OF THE GONADS OF RANA SYLVATICA[1]

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FOUR TEXT FIGURES AND FIVE HELIOTYPE PLATES (TWENTY-TWO FIGURES)

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INTRODUCTION

The study of the development of the amphibian genital glands has attracted the attention of many investigators in the past two decades. And yet we have just learned enough to know what valuable material urodeles and anurans supply for studies in comparative morphology and what promising opportunities they offer for the experimental attack of the

[1] The material for this investigation was collected during a stay at the Osborn Zoölogical Laboratory, Yale University. The microscopical study has been made in the Zoölogy Department of the State University of Iowa. The expenses have been defrayed by the International Education Board and the Committee for Research in Problems of Sex of the National Research Council.

fundamental problems of sex determination and sex differentiation. Even if we do not take into account the secondary sex characters, we must be stricken with surprise by the great variety in the development as well as in the final topography of the gonads of the different batrachian species. Some are as primitive as selachians of the type of Scyllium, while, on the other end, some prove nearly as highly differentiated as mammals, at least for the essential features. Work of recent years has revealed the fact that some of the most common anurans are rudimentary hermaphrodites which eventually may ripen fertile eggs and sperms either in consecutive years, as in the toad Bufo vulgaris, or simultaneously, as in the frog Rana temporaria. In the latter case artificial self-fertilization has proved successful. While such a rudimentary hermaphroditism, occurring in different degrees and shades in numerous species reminds one of the fully developed hermaphroditism of ascidians and some fishes, the separation of the sexes may be found as clear-cut in others as it generally is in mammals.

To this diversity in the morphological appearance corresponds an adequate range of differences in the genetical constitutions. Disregard of these basic facts has led and must lead to embarrassing errors and seeming contradictions. The author intends to publish a series of studies on the descriptive and experimental embryology as well as the genetics of the amphibian sex characters. This first one deals with the normal development of the sex glands of a species with high specialization and pronounced gonochorism. Of all the hitherto described cases, it resembles most closely that of the alpine races of Rana temporaria. However, the sex differences develop much faster and attain at the time of metamorphosis (end of the seventh week) a higher degree than in any other of the hitherto studied species.

MATERIAL

A pair of copulating wood frogs (Rana sylvatica) was caught in a pond at Mount Carmel, near New Haven, Con-

necticut, on April 5, 1926. The next day the eggs were artificially fertilized and the lot used for this study was kept at a temperature which started at 16°C. and became gradually raised to 20°C. This latter was attained at the end of the second week and from then on the temperature was kept constant within the range of $20° \pm 1°$C. The larvae were fed with liver and spinach. The first animals metamorphosed on the forty-fourth and the last ones on the fiftieth day. Ripe eggs and adult ovaries were secured from copulating females, while the adult testis described in this paper was preserved about two weeks after the breeding season had passed. Zenker's fluid (with 5 per cent acetic acid) was used for the fixation of this material.

THE FORMATION OF PRIMORDIAL GONADS

In larvae of 8 mm. total length with free external gills the germ cells are included in the dorsal yolk ridge of the entoderm. This ridge, which separates the two mesoderm plates, extends from the opening of the wolffian ducts into the cloaca, over 1.10 mm. in the cranial direction. It has not been possible, however, to distinguish clearly the germ cells from the archenteron cells. At this time both display the same primordial characters of large globular cells, heavily laden with yolk granules. In just-hatched tadpoles, however, the germ cells become separated from the entoderm by the mesoderm plates which grow toward the medial plane and soon unite, forming the mesentery. The germ cells now lie in a long-stretched-out row in the space between the dorsal root of the mesentery, the two cardinal veins, and the aorta. Thus they occupy exactly the place of the future vena cava.

During the time when the external gills disappear and the gill sacs are formed the two cardinal veins approach each other, and by their final fusion give rise to the unpaired vena cava (compare figs. 1 to 4). Simultaneously the hitherto unpaired row of germ cells splits longitudinally. The two fractions move a short distance in lateral direction. About midway between the mesentery and the wolffian ducts they

reach their final position. As the peritoneum now envelops the germ cells more closely, the primordial gonads become distinct as a pair of straight flat ridges.

Fig. 1 Rana sylvatica, ten-day-old larva, 10 mm. total length (3.5 mm. from nose to anus). Cross-section 0.38 mm. anterior of the ends of the wolffian ducts. *Vc*, cardinal veins; *Wd*, left wolffian duct; *I*, intestine; *M*, mesentery; *G*, gonocytes. Camera lucida, × 375.

Fig. 2 Same larva as in figure 1. Cross-section 0.82 mm. anterior of the ends of the wolffian ducts. The cardinal veins unite to form the vena cava. The germ-cell strand has broken into two, both parts projecting now toward the coelomic cavity, thus forming a pair of germinal ridges. Camera lucida, × 375.

In a tadpole of 10 mm. length (3.5 mm. body length) and just-closed gill sacs we can observe different phases of the last-described processes. As the differentiation proceeds in craniocaudal direction we find paired gonads projecting into the coelomic cavity in the anterior part (fig. 2) and the single median row of germ cells at the posterior end of the sex region (fig. 1). The whole sex region is considerably shorter than was the yolk ridge of the entoderm in 8-mm. larvae. It extends now over the region from 0.35 mm. to 1.02 mm. anterior of the cloacal opening of the wolffian ducts. It is interesting to note that the left gonad is somewhat longer (0.08 mm.) than the one on the right side. This asymmetry is caused by the attachment of the ligamentum suspensorium hepatis to the left side of the base of the mesentery which proves an obstacle for the development of the anterior end of the right gonad (fig. 3).

Somewhat older tadpoles (total length 14.5 mm., body length 5.5 mm., fourteen days) possess what may be designated the typical primordial gonads. Though the animal has grown considerably, the gonads have become much shorter. They lie between 0.79 mm. and 1.20 mm. anterior to the cloacal openings of the wolffian ducts. In consequence of such a contraction in the longitudinal extent, the germ cells become concentrated in the central portion where, at this time, one single cross-section may contain five or more of them. These germ cells are in way of transformation into the characteristic gonia: the cytoplasm becomes more homogeneous as the yolk and pigment granules dissolve. The nuclei grow considerably. Their chromatin-net, while spreading out, becomes very fine and loses gradually its affinity to hematoxylin. Each germ cell is surrounded by a follicle. The center of the gonad is occupied by some mesenchyme cells. The peritoneum covers now almost the whole surface of the gonad, which thus becomes isolated from the somatic trunk.

THE UNDIFFERENTIATED GONAD

At the end of the third week the undifferentiated gonad is at the height of its differentiation. It has become largely

Fig. 3 Same larva as in figures 1 and 2. Cross-section 1.01 mm. anterior of the ends of the wolffian ducts. This section cuts through the center of the spiral of the intestine and through the liver. The portal vein (open space in the drawing) ascends through the mesentery and, turning to the right side, passes through the suspensorium hepatis into the liver. This arrangement leaves no space for the development of the right gonad, while the left one (*g*) regularly extends thus far in anterior direction. Camera lucida, × 50.

separated from the inner body wall to which it is attached only by the mesogonium. This latter is equivalent to the mesentery. It is formed by the two sheets of the peritoneal envelope of the gonad and a few scattered cells of connective tissue between them. It contains the blood vessels and nerves

supplying the gonad and also, at a somewhat later stage and in the male only, the vasa efferentia connecting the testis with the mesonephros.

In longitudinal direction the gonad exhibits now a differentiation into three sections (fig. 5). The main part in the center is the proper germ gland, while the other two are merely sterile appendages. The one at the anterior end may

Fig. 4 Rana sylvatica (no. 3) larva, fourteen days old, 14.5 mm. total length (5.5 mm. from nose to anus); noticeable buds of hind limbs. Cross-section through the typical primordial gonad. *A*, aorta; *Vc*, vena cava; *Wd*, left wolffian duct; *Mn*, blastema of mesonephros; *Gc*, germ cells. Camera lucida, × 375.

be called the progonad. At this time it consists of a compact mass of small mesodermal cells with relatively large basophile nuclei. Occasionally single germ cells are inclosed. This progonad later on gives rise to the fat-body. The first fat-vacuoles have been found in twenty-eight-day-old animals.

The appendage at the hind end consequently is called the epigonium. It essentially consists of a narrow fold of the peritoneum, though it often contains scattered germ cells and some mesenchyme also. As a rule, this epigonium does not

change its character during the further development and is still found in adult animals of both sexes. The few germ cells will disappear soon. Sometimes the epigonium produces a small posterior fat-body.

Our further descriptions will almost exclusively deal with the fertile central section of the gonad—the future germ gland. At this time it mainly consists of a tube which has developed from the preceding stage by the appearance of the primary gonad cavity (fig. 6). Strictly speaking, this is not a cavity, for it is filled by a mesenchymatic tissue of very large cells containing a transparent jelly. The germ cells have become smaller, as the yolk granules dissolve rapidly. They are arranged in a single layer along the peritoneum, thus forming, together with their follicles and some stroma cells, a primitive peripheral germinal epithelium.

At this stage also the primordial *rete cords* have appeared. They are solid plugs which have formed in the hilum region of the gonad by the aggregation of single round cells. These round cells originate from the blastema of the mesonephros, from which they split off and move toward, and finally enter into, the gonad. The rete cords cause a fairly regular sequence of alternating knots and constrictions of the gonads. This condition may be designated as *gonomery*, each single segment representing a *gonomere*.

Thus, the germ gland is composed of at least four kinds of cells of different origin: Peritoneal cells, germ cells, connective-tissue, and rete cells. It consists of two main formations, 1) the germinal epithelium forming the cortex and, 2) the rete cords forming the medulla of the undifferentiated sex gland.

SEX DIFFERENTIATION

The sex differentiation starts at the end of the third week with characteristic changes in the medulla as well as in the cortex.

In some animals the rete cords grow but very slowly and become hollow in their distal part (secondary gonad cavity).

At the same time the germinal epithelium thickens rapidly and some of the germ cells soon enter the prereduction stages. Gonads displaying such changes develop into *ovaries* (fig. 7).

On the other hand, there are animals in which the rete cords grow rapidly but remain compact. At the same time the primitive germinal epithelium disintegrates. The gonia, together with their surrounding follicle cells, migrate toward the center of the gonad and become attached to, or embedded in, the rete cords. At the end of this process the former germinal epithelium is reduced to the simple peritoneal surface epithelium. Gonads of this type will develop into *testes* (fig. 8).

In previous publications ('14) the writer has used the term sex cord (Sexualstrang) for what we call rete cord in this paper. The former term has been adopted by several authors, though the designation of rete cord is preferable, because this structure transforms later on into the rete apparatus; furthermore, the name of sex cord has been used (mainly in mammalian embryology) for the cell rows which in the young testis are formed during the migration of the gonia from the germinal epithelium to the medulla.

In amphibians the gonia move singly or in small groups of two or more, but relatively seldom we observe distinct cell rows. Nevertheless, it is obvious that these migrating gonia correspond to the sex cords of the mammalian testis.

THE DEVELOPMENT OF THE OVARY

In the ovaries large numbers of ovocytes are formed. Within these the ovogonia first multiply by a succession of three or four mitotic divisions and then transform into ovocytes. Meanwhile the secondary gonad cavities widen and thus transform into the ovarial sacs, which are so characteristic for the anuran female gonad. (Figures 7, 9, and 10 show these conditions in cross- and longitudinal sections.)

During the seventh week, when the tadpoles begin to metamorphose, the ovary also transforms into the adult type. The ovocysts of the preceding stage divide into the single ovo-

cytes. This is effectuated by the ingrowth of the follicle cells, which at the end of this process form an envelope around each ovocyte. This envelope is the granulosa of the egg. Now the ovocytes slowly move toward the center of the gonad, and, as at the same time the ovarial sacs become still much wider, the primary gonad cavity disappears almost completely. Some of the mesenchymatic cells transform into a second egg envelope, the theca interna. Others form the connective tissue which fills the spaces between the rapidly growing eggs. The more the eggs enlarge, the more they are protruding into the secondary gonad cavity, so that large parts of the wall of the ovarial sacs virtually become a third egg envelope, which is the theca externa. Figures 11 and 12 show transitional stages of these processes at the beginning and toward the end of the metamorphosis stage. In the ovary of the adult female, each large egg, enveloped in granulosa and theca interna, lies in a sac formed by the theca externa, which with its open side is attached to the peritoneal epithelium (fig. 13). The theca externa therefore does not cover the whole surface of the egg. It really remains to be a part of the ovarial sac, and therefore leaves uncovered the one side of the egg which is connected with the germinal epithelium. By the growth of the ovary the germ layer of the germinal epithelium becomes discontinuous. In the adult female, the ovogonia and young ovocytes form but small groups scattered along the surface of the ovary, which otherwise consists now merely of the simple peritoneal epithelium (figs. 13 and 14). These groups, which I shall designate as *germ patches*, evidently are the exclusive source from which the successive yearly generations of ripe ova arise. In serial sections one regularly finds such germ patches at the basis or in the neighborhood of each ripe egg or empty follicle. As a full-grown female produces from about 300 to 600 eggs, there must be at least some hundred germ patches distributed evenly over the surface of each ovary.

At about the time when the egg is extruded into the body cavity, the large nuclear vesicle bursts and the first matura-

tion division immediately follows. As in Rana temporaria, the egg of sylvatica in the uterus shows the first polar body lying in a cup-like depression at the animal pole and near by the second polar spindle with the chromosomes in the metaphasic arrangement (fig. 15).

After the expulsion of the egg, the empty follicles appear as very compact masses of tissue. As we would expect from the foregoing, the theca externa remains intact while granulosa, theca interna, and peritoneal epithelium are ruptured by the outbursting egg. The granulosa cells with their distinctly basophile nuclei occupy the center of the primitive corpus luteum. The cells of the internal theca acquire a glandular character and produce a secretion by which it separates itself from the granulosa. The theca externa undergoes no transformations, but becomes folded, according to the reduction in size of the follicle (fig. 16).

Some statements concerning the blood supply may be added to this description of the development of the ovary. The arteries and veins enter and leave the ovary through the mesogonium in the number of about four or five pairs. They show a similar metameroid arrangement as the rete cords do, but branch off already in the hilum region. In the young ovaries (figs. 7 and 9) small blood vessels are found distributed through the mesenchyme between the rete cords (ovarial sacs) and the germinal epithelium. Correspondingly in the adult ovary, blood vessels are embedded in the connective tissue between the germ patches and peritoneal epithelium and the ovarial sacs. They generally form a ring around the base of each large egg (figs. 13 and 16) and send numerous fine capillaries into the theca interna (which is the connective tissue of the follicle apparatus), thus producing a very fine vascular network around the eggs.

THE DEVELOPMENT OF THE TESTIS

The early development of the male sex gland is characterized by the formation of the seminiferous tubules and the rete apparatus.

Immediately after the migration of the germ cells which is characteristic for the early male differentiation, as described above, the medulla consists of a compact mass of tissue in which the large germ cells invested in their follicles are easily recognized (fig. 17). It is not possible, at this time, to distinguish rete cells from cells of connective tissue, which eventually might have been included. However, the main part of the connective tissue which had filled the primary gonad cavity of the undifferentiated gland undoubtedly has become converted into the albuginea. During the fifth week a fragmentation of the medulla takes place which results in the formation of the seminiferous tubules (fig. 18). These are very short at this time, develop a covering epithelium which becomes the wall of the tubule, and stay permanently connected with the compact masses of rete cells in the center of the medulla and with the rete cords in the hilum. The rete cords continue to enlarge, growing around the vena cava toward the mesonephros. At this time the testis becomes shorter and spindle-shaped. Its generative parts concentrate at the anterior end, leaving a long epigonium almost void of germ cells (fig. 19).

At about the time of metamorphosis, the rete tubules begin to develop out of the compact masses of rete cells. In the center of the medulla a network of tubules becomes more and more distinct, which communicates with the seminiferous tubules by short tubuli recti and with the mesonephros by the vasa efferentia (fig. 20). The latter derive from the extragonadal parts of the rete cords. Therefore they occur in numbers of four or five. Later, some longitudinal anastomoses complicate this arrangement. The testes of metamorphosed males are extremely short. They are so different from the long ovaries of the females that the sex of dissected animals can easily be identified even without the aid of any lenses (fig. 21).

None of the numerous first-year males of this species showed any signs of germ-cell maturation. The germ cells multiply rapidly, but stay on the stage of the primary spermatogonia.

We have not had an opportunity to examine half-grown wood frogs. The full-grown are relatively small and, as the early stages develop rather rapidly, we may assume that they attain maturity during the third season.

The testis of the adult undergoes cyclic changes through the year which correspond to those described in detail by the author for Rana temporaria ('24). However, the stages are not so distinctly separated. Very different phases of the spermatogenesis can be found in the same testicle. Thus, in a frog killed on May 3, 1926, we find sperms which have not been extruded during the breeding season and all stages of the new spermatogenetic cycle up to synapsis (fig. 22). Primary spermatogonia are scattered all along the walls of the spermatic tubules; some of them are in mitotic division. These spermatogonia never are entirely used in the production of sperms, but some always are spared from ripening. Such undifferentiated gonia have been called 'residual cells' (Swingle, '27). They are the constant source of the subsequent spermatogeneses throughout the entire life of the frog.

There are but few interstitial cells found in the narrow spaces between the seminiferous tubules.

The supply of the testis with blood vessels is essentially the same as that of the ovary. About five pairs of arteries enter through the hilum. They follow the connective tissue and form a very dense network of small vessels and capillaries around the seminiferous tubules. On sections we find them, therefore, between the tubules, where they have to share the narrow space with interstitial cells and strands of connective tissue.

DISCUSSION

The specificity of the germ cells

In his classic monograph on the development of Bombinator, Alexander Goette, in 1875, first reported on observations from which he inferred that the sex cells arise directly from undifferentiated yolk-laden embryonic cells. His descriptions

show clearly that he has seen the unpaired strand of primordial germ cells at the dorsal base of the mesentery and observed the formation of the paired germinal ridges from the very beginning. The citation of this fact by the author in an earlier paper ('14) led Moritz Nussbaum to a rather acrimonious retort in the Anatomischer Anzeiger (December of the same year), in which he not only claims the exclusive priority for the idea of a specific nature of the germ cells, but also pretends to have furnished evidence for the accuracy of this hypothesis. I still believe that to-day the substantial contributions of Goette and Nussbaum toward the problem of the ontogenetic origin of the germ cells in anurans are of but historical interest. The microscopical technic before 1880 was not sufficiently developed to allow these investigators to really trace germ cells and somatic cells with all the scrutiny indispensable in this case. No doubt both have seen the primordial germ cells at a very early stage. But they have not settled the question if there occurs regularly or occasionally a secondary transformation of mesodermal cells into germ cells. Even to-day, microscopical investigation alone seems to be unfitted to settle that problem. The only real success in this field in relatively recent times was B. M. Allen's discovery ('07) that the primordial germ cells of the anurans arise from the embryonic entoderm. Beyond this, we must note that, despite the refined technic, for every paper asserting that there is only one germinal line another one is written to describe the differentiation of secondary germ cells. We mention only the literature on anurans, which, however, gives a true picture of the whole deplorable situation. Bouin ('01) and Dustin ('08) describe the transformation of mesodermal cells into germ cells at different larval stages. Allen ('07) does not find it in the early stages which he examined, and King ('08), studying the complete ovogenesis of Bufo lentiginosus, declares positively that throughout the whole life-cycle there is no such production of mesodermal sex cells. Kuschakewitsch ('10) again finds, besides the primordial entodermal germ cells, secondary ones

which arise from any kind of tissue participating in the formation of the gonads, such as peritoneum, mesenchyme, and rete cords. Witschi ('14) corroborates King, while Gatenby ('16) claims to give definite proof of neoformation of ovogonia in the adult ovary. Swingle ('21) prudently leaves the question open, while Beccari ('24, '26), Bounoure ('25), and Perle ('27), again investigating early stages only, deny any secondary transformation.

There is no question but that in microtome sections, occasionally, cells appear which cannot with certainty be classified either as germinal or as somatic, and they may be then interpreted as transitional cells. No critical mind, however, will easily be convinced by such meager evidence as long as the constant presence of numerous undifferentiated gonia leaves the possibility open of a common origin from primordial gonia. Gatenby, in his above-mentioned paper, claims to have settled the question by his statement that, in the ovary of hibernating females of Rana temporaria, only auxocytes but no ovogonia are present. In spring, new ovogonia arise in the germinal epithelium by transformation of somatic cells. However, the author has made a study of the same species, and in a paper which is in preparation will show that during hibernation ovogonia are present in the ovaries in considerable numbers in October as well as in early February.

In recent time, descriptive morphology has often obtained valuable support by the use of experimental methods. And it seems highly probable that the problem here discussed will be solved in this way, too. The first attempt was made by G. Levi in 1905. He tried to castrate 9-mm.-long larvae of a European toad by cauterizing the region where the germinal ridges lie. Apparently his operations were incomplete and the gonads regenerated. Davenport ('25), Haterius ('27), Parker and Fielding ('27), Hanson and Heys ('27) report on cases of apparently complete ovariotomy followed by regeneration in mice and rats. However, as Hanson says, the general impression is "that the last word has not yet been said on this subject of mammalian ovarian regenera-

tion.'' Reagan ('16) tried to remove the extra-embryonic germ cells in chick embryos. In one case the five-day-old operated embryo had no typical germ cells left, though it possessed some large cells which somewhat resembled gonia. These could be interpreted as a beginning of regeneration out of somatic material. Humphrey ('27) removed unilaterally the primordial germ cells of Amblystoma embryos and in the cases of successful operation has not found any regeneration up to the 290th day. In weighting this experiment, one has to consider that the presence of one gonad may suppress the regenerative stimulus. We know that this is true in female fowls. This may be also the cause of the degeneration of germ cells in incompletely castrated Amblystomas observed by Humphrey.

Coming back to the anurans, we have to mention the experiments with overripe frog eggs (delayed fertilization). Already, Pflüger ('82) and Rich. Hertwig (since 1905) had mentioned that in such cultures the gonads develop abnormally slowly. Kuschakewitsch ('10) made an extensive study leading him to the conclusion that no primordial germ cells are given off by the entoderm, but that relatively late (prevailingly after metamorphosis) spermatogonia differentiate out of small cells of the rete cords (the whole offspring of such cultures therefore being male). The author (Witschi, '14) expressed some doubts regarding the correctness of Kuschakewitsch's observations. He agrees in the observation that the normal type of large gonia with oxychromatic nuclei is completely lacking in about half of the animals of an experimental group. However, among the small cells with basichromatic nuclei some contain unusually large amounts of pigment granules. It could be demonstrated that these represent the type of small cells which later, in some cases several months after the metamorphosis, transform into germ cells. About half of the animals possessed both normal germ cells and pigmented cells. In this case the latter enter the germinal ridges somewhat later than the normal ones. Recent studies (unpublished so far) show that

both types arise from the entoderm, but the pigmented cells migrate more slowly from the very beginning. It is quite obvious that Kuschakewitsch overlooked these modified germ cells, which must have occurred at least in some of his animals, too. Notwithstanding this criticism, we must admit a possibility that in extreme cases overripeness might completely suppress the migration of the entodermal germ cells, for, as just mentioned, it certainly delays their migration and in some cases the pigmented cells were fewer than the germ cells in a normal gonad.[2]

Considering the known facts altogether, we are convinced that, in the normal life-cycle, no secondary (mesodermal) germ cells are formed. The question if in some experiments removed germ cells can be replaced by mesodermal elements needs further investigation before it can be settled.

Gonomery and asymmetry

The gonomery which was described above is apparently derived, phylogenetically, from the primitive metameric condition which still seems to persist in Gymnophiona. Even in some anurans, as for instance in Pelobates fuscus, this segmentation is very conspicuous, young gonads showing about a dozen distinctly separated knots. Only the anterior ones develop into the final gonads, the ovary extending over about ten, and the testis over five to six segments. In Pelobates, as well as in Rana, the gonomeres are much shorter than the metameres of the muscular system. In 20-mm.-long larvae of the wood frog they are less than half as long. It is very probable that this contraction is a consequence of the general contraction of the genital anlagen. In

[2] Recently, Bounoure ('27) has published two short notes on the development of overripe eggs of frogs and toads. Apparently he has not succeeded in getting a high-grade overripeness, though he also finds that the primordial germ cells are densely pigmented and migrate more slowly than normally. He does not realize, however, that with such statements he exactly repeats what the author described in 1914 (pp. 70 to 73, figs. 59 and 60), but, on the contrary, claims to give evidence that Witschi's statements on the mesodermic formation of germ cells in overripe material are all wrong!

the males of both Pelobates and Rana this process carries much further than in the females, as one may state by comparing figures 10 and 19 representing ovary and testis of sylvatica at the same age and both including five or six segments. Ovaries and testes are much shorter in anurans than in urodeles, and this fact apparently is correlated with the reduction in length of the body axis in the former group.

It is of some interest to see that this shortening of the body in connection with the right-sided attachment of the liver causes an asymmetry of the gonads. In early stages the liver prevents the right gonad from extending as far cranially as the left one does (fig. 3). This affects but slightly the development of the fertile germ gland; however, the fat-bodies are always much smaller on the right side (fig. 21). Stieve ('24) holds that the same two factors caused the size reduction of the right testis and the almost complete disappearance of the right ovary in birds. Our observations on frogs cannot but support this idea.

Sex differentiation and sex determination

We have followed as closely as possible the stages of sex differentiation. However, pure observation of the normal developmental processes proves absolutely unsuited to elucidate the question how the alternative between the two possible sexes is decided, i.e., how the sex is determined. Only experiments can possibly reveal what part is played by hereditary factors and which are the physiological realizators to stimulate the morphological differentiation. The next studies will deal with some experiments approaching the determination problem in this sense. The trend of the present paper was, 1) to determine especially the type of sex differentiation in Rana sylvatica, which proves to be clear-cut gonochoristic, and, 2) to establish once more that the general sex characteristics in anurans are, for the female: *a*) the cortex is the permanent site of the germ cells; *b*) the germ cells transform into ovocytes and eggs; *c*) the rete cords of the medulla transform into ovarial sacs which are not connected

with the mesonephros; for the male sex: *a*) the cortex becomes reduced to the peritoneal epithelium because of the emigration of the germ cells; *b*) the germ cells transform into spermatocytes and sperms; *c*) the rete cords of the medulla give rise to the seminiferous tubules, which contain all germ cells, and to the rete tubules, which constitute an efferent system of the testis connected with the mesonephros.

With these basic features in mind, it will be possible to understand the changes in the sex glands involved by experimental factors and to analyze the hermaphroditism of young and adult specimens which is normally observed in certain races of frogs and toads.

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PLATE 1

EXPLANATION OF FIGURES

5 Rana sylvatica (no. 8) larva, twenty-two days old, 20 mm. total length (8 mm. nose to anus; 0.3 mm. buds of hind limbs). Left undifferentiated gonad. *p*, progonad (fat-body); *g*, germ gland; *e*, epigonad; *gc*, gonocyte within the fat-body. Through the hilum (left side in the drawing) six rete cords enter the germ gland. × 100.

6 Same animal as in figure 5. This cross-section through the left undifferentiated germ gland shows the germinal epithelium with large primordial germ cells, the primary gonad cavity with some mesenchyme cells and a primordial rete cord projecting from the hilum into the gonad cavity. Photomicrograph, × 300.

7 Rana sylvatica (no. 16) larva, twenty-eight days old, 29 mm. total length (11 mm. nose to anus; 1.1 mm. buds of hind limbs). Cross-section through an early stage of a differentiating ovary. The germinal epithelium contains still primordial germ cells near the hilum, but ovocytes in its crest. Near the distal end of the rete cord a secondary gonad cavity has appeared. The compact piece of tissue in the primary gonad cavity, between the rete cord and the crest of the germinal epithelium, is a section from a blood vessel. Photomicrograph, × 200.

8 Same animal as in figure 5. This cross-section through the right germ gland shows that in one gonomere sex differentiation in the male sense has just started. The rete cord is very compact. In two places germ cells leave the germinal epithelium and join the rete cord. Photomicrograph, × 400.

9 Rana sylvatica (no. 30) larva, thirty-three days old, 32 mm. total length (13.5 mm. nose to anus; 2.5 mm. hind limbs). Cross-section through a young ovary. In the primary gonad cavity four cross-sectioned small blood vessels can be seen. Photomicrograph, × 200.

10 Rana sylvatica (no. 37) larva, thirty-eight days old, 36 mm. total length (15 mm. nose to anus; 3.5 mm. buds of hind limbs). Frontal section through the young ovary, showing the metameroid arrangement of the six ovarial sacs. The epigonium with the characteristic attachment to the vena cava. Photomicrograph, × 110.

PLATE 2

EXPLANATION OF FIGURES

11 Rana sylvatica (no. 55) larva, forty-four days old, 39 mm. total length (15 mm. nose to anus; 5.5 mm. hind limbs). This cross-section through the ovary of a full-grown larva shows the rete ovarii with its small solid stalk in the hilum and the endothelium forming the ovarial sac. Some ovocytes have grown considerably (auxocytes). Photomicrograph, × 200.

12 Rana sylvatica (no. 57). Fifty-day-old frog with tail rudiment. Cross-section through the left ovary. All stages of germ cells from ovogonia to relatively large auxocytes are present. Photomicrograph, × 200.

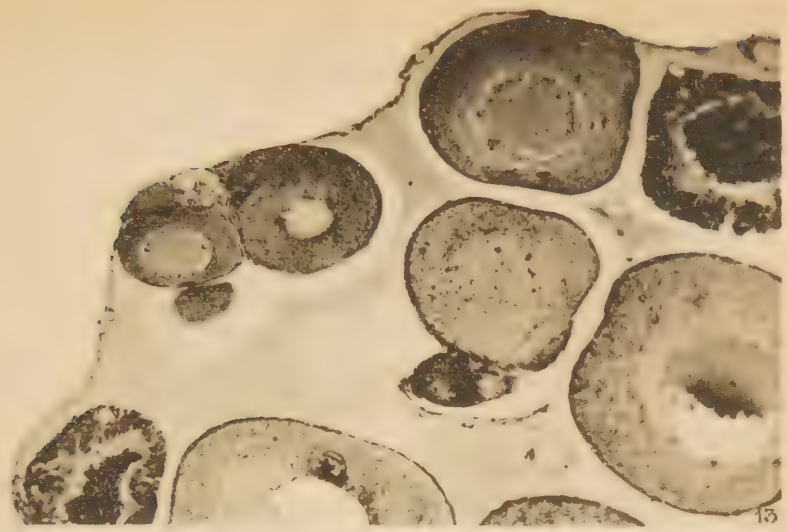
PLATE 3

EXPLANATION OF FIGURES

13 Rana sylvatica, adult female. Section of an ovary shortly after ovulation. At the upper left, a germ patch with ovogonia and ovocytes. At the upper right, an ovocyte which was destined to ripen after a further year, showing the typical attachment to the peritoneum; peritoneum and ovarial sac can easily be distinguished, especially at the right side of the picture, where a larger blood vessel lies between them. *F*, empty follicle. Photomicrograph, × 100.

14 The germ patch of figure 13 at a higher scale. At the top, four ovogonia with light cytoplasm. Below them, ovogonia and early ovocytes in way of producing a new generation of eggs. In the deepest layer two auxocytes, the left one showing the three egg envelopes. Photomicrograph, × 300.

15 Rana sylvatica. Section through the animal pole of an egg from the uterus. On the right side, the cup with the first polar body; on the left, the second maturation spindle. Photomicrograph, × 600.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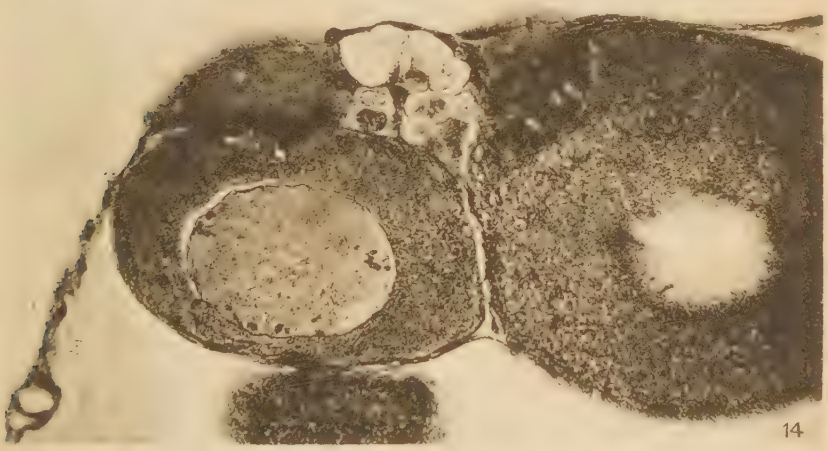
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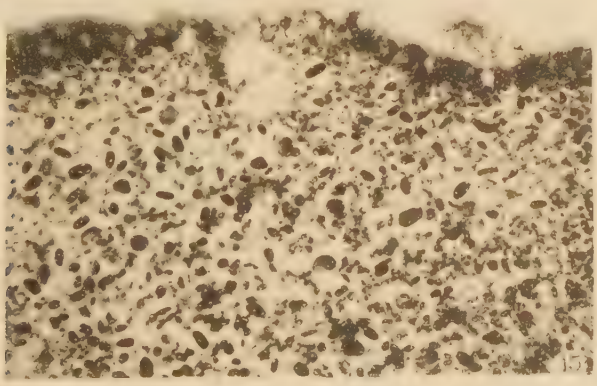
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PLATE 4

EXPLANATION OF FIGURES

16 Rana sylvatica. Section of an ovary after ovulation, showing the empty follicles (rudimentary corpora lutea). Photomicrograph, × 100.

17 Rana sylvatica (no. 18), twenty-eight-day-old larva, 28 mm. total length (12 mm. nose to anus; 1 mm. buds of hind limbs). Early stage of testis. All germ cells are embedded in the distal part of the rete cord. Photomicrograph, × 300.

18 Rana sylvatica (no. 25), thirty-three-day-old larva, 33 mm. total length (13.5 mm. from nose to anus; hind limbs, 2.5 mm.). Differentiation of primordial seminiferous tubules. Photomicrograph, × 200.

19 Rana sylvatica (no. 33), thirty-eight-day-old larva, 37 mm. total length (15 mm. from nose to anus; hind limbs, 4.5 mm.). Frontal section, showing the contraction of the germinal part of the gonad, leaving a long epigonad at the posterior end (compare with the corresponding ovary in fig. 10). Photomicrograph, × 110.

20 Rana sylvatica (no. 58), fifty-day-old frog with tail rudiment. Testis with distinct seminiferous tubules and vas efferens. Photomicrograph, × 200.

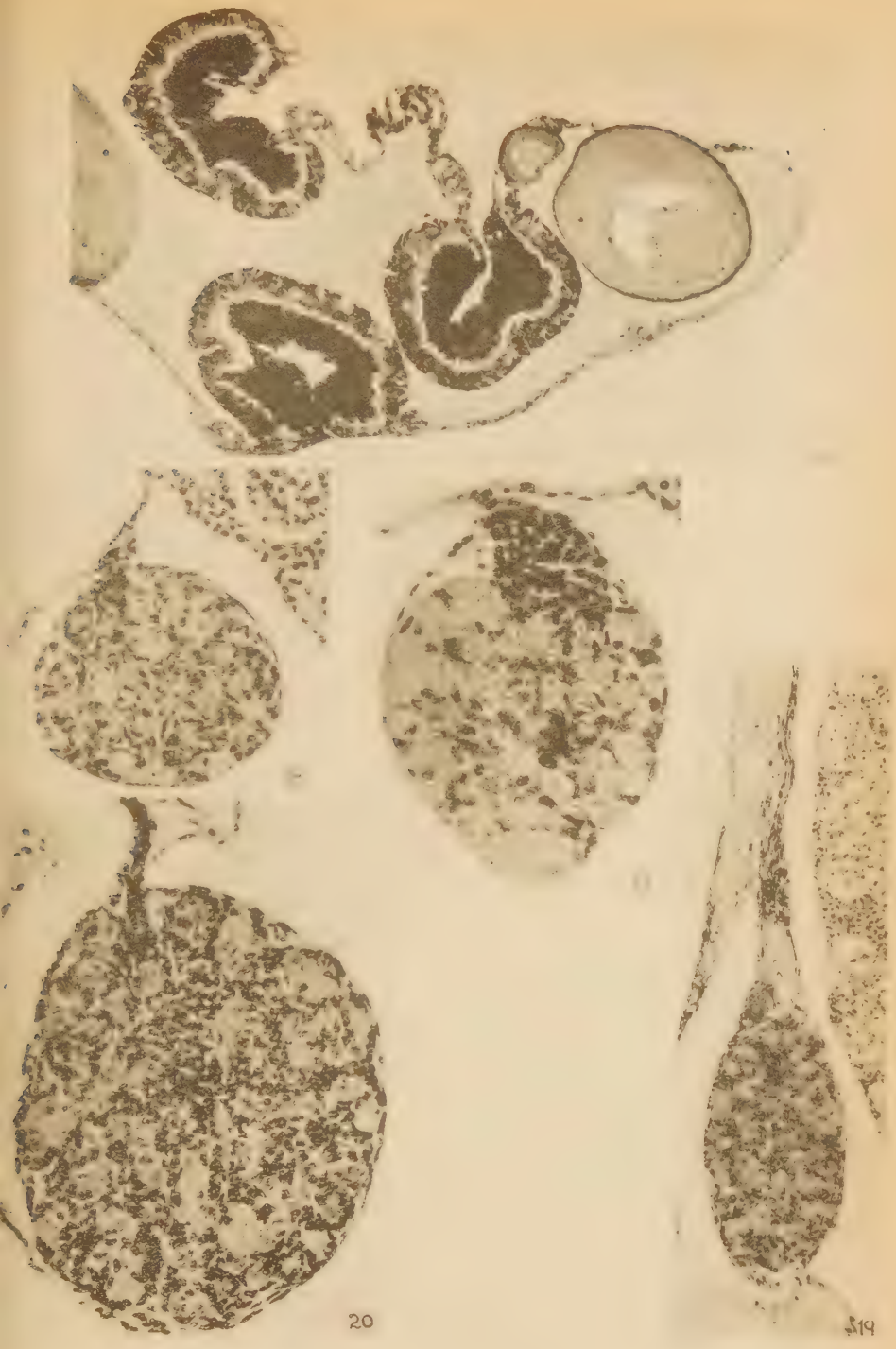
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PLATE 5

EXPLANATION OF FIGURES

21 Rana sylvatica (nos. 57 and 58), at the end of metamorphosis. Kidneys and gonads of a female and a male. On the left side, the long stretched ovaries with lobes still indicating the gonomery. On the right side, the short testes. Note that in both sexes the fat-bodies on the left are several times larger than the ones on the right side. Photomicrograph, × 10.

22 Rana sylvatica. Adult male one month after the breeding season. Part of a section through the testis, showing the proximal half of a seminiferous tubule and its connection with the efferent tubules. Considerable masses of degenerating sperms in the lumen of the tubule. Along its walls, primary and secondary spermatogonia and spermatocytes up to the synapsis stage. Between the tubules, sparse interstitial cells and blood vessels. Photomicrograph, × 300.

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STUDIES ON SEX DIFFERENTIATION AND SEX DETERMINATION IN AMPHIBIANS

II. SEX REVERSAL IN FEMALE TADPOLES OF RANA SYLVATICA FOLLOWING THE APPLICATION OF HIGH TEMPERATURE[1]

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FIVE HELIOTYPE PLATES (TWENTY-SEVEN FIGURES)

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PLAN AND ARRANGEMENT OF THE EXPERIMENT

It sometimes occurs that in the early differentiation of the testes some of the primordial germ cells fail to reach the medulla in proper time. They still lie in the peripheral germinal epithelium at a time when in the ovaries the first set of ovocytes enter the prereduction stages. It is interesting that in these testes the cortical germ cells transform into ovocytes exactly as they do in the ovaries. Consequently,

[1] The material for this investigation has been secured during a stay at the Osborn Zoölogical Laboratory, Yale University. The microscopical study has been made in the Zoölogy Department of the State University of Iowa. The expenses have been defrayed by the International Education Board and the Committee for Research in Problems of Sex of the National Research Council.

such a gonad is hermaphroditic for a short time (fig. 1). However, very soon, the ovocytes degenerate and disappear completely. If we consider that all germ cells of one individual have the same—in this case the male—genetical constitution, it becomes obvious that cortex and medulla must somehow be responsible for the differentiation in opposite directions. From similar facts observed on other material the writer ('21) has inferred that cortex and medulla produce sex-specific morphogenetic substances.

Hence in the cortex would be localized the female-differentiating system and in the medulla the male-differentiating system. As hermaphroditism in amphibians, as in higher vertebrates, never is a stable physiological condition, we must assume that the differentiators act as antagonists. Either one, apparently, is apt to suppress the development of the other one under certain circumstances.

In an effort to support this heuristic hypothesis, some experiments have been planned. This study deals with the effect of high temperature upon the germ glands of Rana sylvatica tadpoles after the sex had already become differentiated.

The development of the gonads of the normal controls has been described in the preceding study. The sexes differentiate during the fourth week. At the end of the fifth week, the experimental lot was separated from the normal one and its temperature raised to $32° \pm 2°C$. This is approximately the maximal temperature at which tadpoles of this species can be kept for a long time. They do not even support this temperature too well, as indicated by their slow growth and the delay in metamorphosis. The latter started three days after that of the normal lot. Two weeks after the end of the metamorphosis of the normals, a considerable fraction of the heat animals still had not transformed. Up to the thirty-fourth day when the temperature was raised, thirteen females and fifteen males were found among twenty-eight controls. Mortality does not interfere with this experiment, as the few animals which died after the rise of temperature have been preserved and are included in the study.

THE REACTIONS OF THE TESTES

The further development of the male gonads is not essentially different from that of the normals. The fat-bodies become extremely small and at the end of the experiment the testes also are considerably reduced (compare fig. 2 with fig. 21 of the first study). The shape of the latter is the normal one, but their dimensions are reduced to about two-thirds in every direction, so that the volume of the high-temperature testes is about one-third of the normal testis. On sections one encounters not infrequently degenerating spermatogonia; however, not in a sufficient number to explain wholly the size reduction of the testes. The spermatogonia are of normal size and, as the seminiferous tubules differentiate in a typical way, the size reduction must be prevailingly due to a retardation in growth (fig. 3).

THE REACTIONS OF THE OVARIES

The reactions of the ovaries to the heat treatment are much more complex and more important.

1. Degeneration of the ovocytes

At the time when the temperature was raised the ovaries contained large numbers of ovocytes in the synapsis stage, besides a considerable number of undifferentiated ovogonia (compare fig. 9 of the first study). Two weeks later, these young ovocytes have completely disappeared (fig. 4). The ovogonia are still present, typically scattered along the periphery of the gland. The deeper parts of the germinal epithelium contain auxocytes. These, however, are in way of degeneration. The most characteristic feature of this degeneration is the appearance of extremely large and intensely chromophile nucleoli—generally one in each cell—with a large central vacuole. The ovoplasm has lost most of its normal basophilia and contains relatively coarse granules. Figures 5 and 6 represent the typical conditions in the normal and in the experimental ovary. In the first one a germ patch with nests of young ovocytes indicates that the egg formation is

still rapidly going on. The large auxocytes in the deeper layer of the gland contain nuclei with a very fine chromatin-net and a considerable number of evenly distributed small nucleoli. The eggs of the experimental animal (fig. 6) are much smaller—which explains the rudimentary aspect of these ovaries (fig. 2). They show the pathological features mentioned above.

From these observations it becomes obvious that, shortly after the rise of temperature, the further production of ovocytes has stopped. The ones formed before passed from the synapsis into the auxocyte stage. But soon their growth assumed an abnormal character and, after fifteen days of heat treatment, the ovocytes without exception are found in full disorganization.

Within two more weeks the ovocytes disappear completely. Figure 7 gives a picture of the odd transformations which may result from such a process. The ovarial sac retains an approximately normal size, while the germinal epithelium—reduced to the peritoneum, some ovogonia, and a sparse connective tissue—shrinks until little more than a thin membrane is left.

2. The exuberant growth of the rete cords

The last-described condition, however, has been observed in a few cases only, and even in those the larger part of the gonads follows a more common type of reactions. Soon after the degeneration of the auxocytes has begun, usually the walls of the ovarial sacs thicken and exhibit more and more a tendency for copious growth. This is well marked already in some females of the forty-ninth day (fifteen days of heat treatment), as can be seen in figure 8. Sixty-three-day-old animals with almost completely destroyed ovocytes show distinctly that this exuberant growth finally fills not only the cavities of the ovarial sacs, but also proceeds peripherally, replacing the deeper layers of the germinal epithelium (figs. 9 to 12).

3. The differentiation of the rete apparatus and of the seminiferous tubules

If we remember that the ovarial sacs developed out of the rete cords of the undifferentiated gonad and therefore are homologous with the medulla of the testis, we realize that the described growth may be the first step in the development of a male sex gland out of an ovary. And this proves to be the case. Closer study of the photomicrographs reveals that the growth of the ovarial sacs results not merely in a production of an unorganized mass of tissue. Already in figure 8 we observe some tendencies of tubule formation in different places of the thickened wall. Figures 10 and 11 illustrate clearly the transformation of parts of the ovarial sac into narrow efferent ducts. This process has come to an end in an animal twelve days older from which is taken photomicrograph 13. The lumen of the ovarial sac has become reduced to a very fine efferent duct leading from the center of the gonad to the hilum and through the mesogonium toward the median edge of the mesonephros. Being somewhat curved, its proximal part shows only as a cross-section in the mesogonium on figure 13.

While in this way the central parts of the ovarial sacs transform into the rete apparatus, the peripheral layers form small buds (fig. 9) which soon grow out toward the periphery of the sex gland (figs. 10, 11, 12). Cell plugs are formed in this manner and the nuclei show a tendency to arrange themselves along the surface, leaving a central space filled with a light cytoplasm only (fig. 10). These plugs are, in fact, primordial seminiferous tubules which lack but the spermatogonia to be considered as typical (figs. 13, 14).

From a purely morphological point of view it is worth while to stress the fact that not only the rete tubules, but the seminiferous tubules, also, derive from the rete cords. It is possible, of course, that some mesenchymatic elements join the rete cords. It possibly could be that the thin epithelium which covers the outside of the seminiferous tubules (figs. 11, 12, 14) arises from that source. Yet the fact to put emphasis

upon is the non-participation of the germinal epithelium. Generally, it is assumed that the proliferation of the germinal epithelium—often designated as the 'first proliferation'—which in the normal development of the vertebrate testes carries the spermatogonia into the medulla, produces the primordial seminiferous tubules (sex cords). This is certainly not true in our experimental material and it is probable that a reinvestigation of other vertebrate groups would prove that in a general way the seminiferous tubules arise from the rete blastema.

4. The migration of the primary ovogonia into the seminiferous tubules and their inversion into primary spermatogonia

What in the preceding paragraph have been designated as seminiferous tubules are, in fact, only the basic structure of such and consist of the epithelial cells which later on will form the walls of the tubules. In no case is there any indication of germ-cell formation inside of them, but the spermatogonia arise from the germinal epithelium exactly as they do in the normal testis. We have here a particularly clear case to demonstrate the specific nature of the germ cells.

The heat treatment causes the degeneration of the secondary ovogonia and the ovocytes only, while the primary ovogonia are spared. In a few instances primary ovogonia have been seen growing and transforming into giant cells (fig. 26) which, no doubt, some time later will degenerate. However, as a rule, the ovogonia stay intact, scattered along the peritoneal epithelium. Toward the hilum they are more numerous than on the distal crest of the gonad (figs. 7, 10, 11, 12, 13).

As long as the ovarial sac remains thin-walled, the ovogonia stay closely attached to the peritoneum. However, this rudimentary germinal epithelium disintegrates soon after the beginning of the medullary growth. In some cases, as in the one represented by figure 15, the ovogonia start to move toward the center of the gonad even before seminiferous tubules have become differentiated. Far oftener, however,

this occurs somewhat later (fig. 16). The germ cells first move in the narrow spaces between the seminiferous tubules (figs. 16, 17), but soon become incorporated into their walls (figs. 18, 19). Figure 20 shows a gonad in which a few germ cells still lie outside the tubules, while a larger number are inside. This gonad differs from the normal testis mainly by the much lesser number of germ cells. It is evident that this gonad really must be considered as a testis. It may be called the next to the last step on the way of the transformation of the ovary into a testis. The very last one could no longer be distinguished from the normal testis. In some ovaries the heat reaction apparently commenced at an earlier stage than in the above-described series. The difference must be due to the fact that, resulting from the general variability observed in any group of animals, some ovaries were a little behind in development at the time when the temperature was raised. Photomicrographs 21 to 24 and 25 show that essentially the same transformations take place. Only exceptionally single auxocytes may be found in this group, as seen in figure 21. Figures 22 and 23 are some of the best illustrations of the transformation of ovarial sacs into rete and seminiferous tubules. In the gonad represented by figure 24 the general topography of a testis is nearly attained, though the ovarial sac has not yet completely disappeared, but shows as a slit in the center. Figure 25 shows a cross-section through the smallest gonad of this series. One ovogonium has remained at the periphery. The other ones have entered the seminiferous tubules and transformed into spermatogonia.

SEX REVERSAL AS AN EFFECT OF HIGH-TEMPERATURE TREATMENT

Sex reversal of the females as the general result of the heat experiment becomes more evident when the single cases are classified as they appear in table 1. In case of unequal development of different sections of the gonads of one animal the condition in the most advanced gonomere has been considered for the classification. There are three distinctly cir-

cumscribed transitional stages of the transformation process characterized by, 1) ovocyte degeneration, 2) medullary growth and, 3) transformation of ovogonia into spermatogonia. It seemed advisable to divide the last class into three, according to the number of spermatogonia present in the medulla, so that the table contains five classes of progressive stages of sex reversal.

The three points of general interest arising from this table are, 1) the total absence of typical females after the second week of heat treatment; 2) the progressive transformation of

TABLE 1

AGE IN DAYS	TEMPERATURE, 20°C.		TEMPERATURE, 32°C.						
				1	2	3	4	5	
	♀	♂	♀	No synaptene, auxocyte degeneration	Medullary growth	Seminiferous tubules, few spermatogonia	Seminiferous tubules, more spermatogonia	Nearly typical male	♂
34	13	15	Rise of temperature						
38	7	4							
44	6	7							
49	18	26		3	2				3
51	56	44			7	1			5
56					2	1	1		10
60						4			2
63					1	7	2	2	21
67						3	15	2	21
Total,	100	96	53						62

the female part of the experimental group toward the male type; 3) the ratio of 0 females: 53 transformers: 62 males.

It is evident that the fifty-three transformers are genetically females. Their number is considerably lower than that of the males. It is not impossible that among the sixty-two males there are a few completely transformed females, which of course could not be identified as such. The theoretical expectation would call for 57.5 of either sex with a probable error of 3.6. The observed deviation is not too unusual.

Further on, we must consider that the experimental group has been split off from the normals, which show a small excess of females (100 females: 96 males). Supposing that the transformers comprise all the genetical females, we can reunite the two groups. The resulting ratio of 153 females: 158 males would be very close to the theoretical expectation. Statistics, therefore, does not decide the question whether or not there are genetical females among the sixty-two morphologically typical males.

It would be of a certain interest to know whether the heat-effect is reversible. Our experiment does not contribute toward this question. Though basing on the actual knowledge we have on sex differentiation in amphibians, I am inclined to assume that the turn toward the testicular type is irreversible after the medullary growth once has become stimulated.

DISCUSSION

As early as in 1914, the author reported on sex reversal in Rana temporaria caused by cultivation under high temperatures. However, that experiment differs from the one here presented in that the high temperature was applied throughout the whole embryonic and larval development. The larvae were not vigorous, and the temperature was kept at 27°C. only. There was a considerable mortality and only in a part of the females had sex transformation set in when they were preserved two weeks after metamorphosis. Though the general effect of heat application was well established by this experiment, the case was not favorable for a detailed analysis.

The first point of interest in the present experiment is the fact that the sex transformation does not start with the destruction of ovogonia or ovocytes, but with the suppression of further ovocyte formation. This really means that the female differentiator does not work any more. The ovogonia (with the exception of the few starting giant growth) are not injured at all. They even continue to multiply by mitotic division (fig. 10). Likewise, the young ovocytes (synapsis stage) present at the onset of the experiment (fig. 9 of the

first study) do not degenerate, but develop into auxocytes, which form the characteristic constituent of the germinal epithelium at the end of the second week (figs. 4, 6, and 8). We must conclude, then, either that the female morphogenetic substance is destroyed by the heat or that its elaboration is suspended. In either case there is a break in the normal chemical process. The germ-cell follicles (granulosa), which most likely are the producers of such substances, remain morphologically unchanged.

Secondly, we wish to emphasize the fact that the medullary growth is not hampered by the presence of auxocytes. A comparison of such figures as 7, 8, and 9 makes clear that there is no correlation of any sort between the amount of auxocytes and the beginning or the rate of the medullary growth. In such cases as animal no. 4 (fig. 8), evidently, the hypertrophy must have started already while the ovocytes were still growing. The cessation of inhibition of medullary growth thus coincides in time with the cessation of ovocyte formation. This suggests that both are controlled by the same factor, i.e., by the hypothetical female morphogenetic substance or the female differentiator.

The third fact we wish to put stress upon is the formation of seminiferous tubules prior to the appearance of spermatogonia. This supports not only the view expressed by the author in earlier publications ('21, '25) that the germ cells do not act as the leading part in sex differentiation, but shows distinctly that even the development of somatic sex characters is independent of the presence and physiological activity of germ cells of the same sex. The same conclusion can be inferred from Domm's ('27) experiments in ovariotomy in the fowl. While it appeared probable that the female morphogenetic substance is produced in the follicles of the germ cells, we must assume that the male one is elaborated by some elements of the rete cords. A diagram (fig. 27), reproduced from an earlier paper of the author ('14), will illustrate, then, the sex value, so to speak, of the different parts of the gonad. The peritoneum seems to be indifferent.

It contains not only the undifferentiated germ cells of the undifferentiated gonad. Even the gonia of the young and the adult ovary, though called ovogonia, stay undetermined as long as they are embedded in the peritoneum. Peritoneal gonia really are bipotential, as evidenced in sex reversal of young and adult females. The deeper layers of the germinal epithelium include the female-differentiating system (*F*), which causes germ cells to differentiate into ovocytes and inhibits the growth of the rete cords. The transformation of the rete cords into ovarial sacs must be linked with the same activity. The rete cords in the center of the sex gland contain the male-differentiating system (*M*). If it becomes active, it causes the germ cells to migrate from the cortex into the medulla of the gonad and to transform into spermatogonia. It suppresses all female differentiations and causes the formation of seminiferous tubules.

The undifferentiated gonad contains the primordia of both cortex and medulla. In the first period of our experiment, evidently, some hereditary factors decided whether the first or the second one of these anlagen should develop, thus producing females and males in practically equal numbers. It certainly is a surprising fact that, in the second period, after heat destruction of the female differentiator, the medulla almost immediately starts to grow, proving that the male differentiator has become active. This, no doubt, constitutes a case of compensatory hypertrophy in the sense of F. R. Lillie ('27). It is a fortunate condition that investigations with such different materials as amphibians and birds lead to almost identical conclusions. In fact, the only difference between Lillie's and the author's theories of sex differentiation consists in the use of the terminology of the hormone hypothesis by the former (Lillie, '27, pp. 178, 185), where we prefer merely to speak of morphogenetic substances or differentiators. We may recall that we do so because at least some of the reactions are strictly localized, as proved by the simultaneous differentiation of eggs in the cortex and of sperms in the medulla of hermaphrodites observed and described at several instances (Witschi, '25).

Evidence is accumulating that, in all vertebrates, or at least in those with a connected urogenital system, the suppression of the normal development and functions of the ovarian cortex releases the compensatory growth of the medulla and its subsequent differentiation into rete and seminiferous tubules. Kuschakewitsch ('10) was the first one to describe such a case in his studies on the development of overripe frog eggs. His figures 70, 71, 54 correspond closely to our figures 9, 14, and 19. His case is different in so far as no typical ovarian germinal epithelium had developed at all, its formation being suppressed from the very beginning. We referred already to the ovariotomy experiments in fowls, in which the removal of the left ovary is followed by the medullary growth in the right rudimentary gonad.[2]

In mice sterilized by x-ray treatment, Brambell and his collaborators ('27) observe the proliferation of cords which resemble the so-called spermatic cords described in the ovaries of "inbred rabbits by Hammond, and of free martin cattle by others" (quotation from the author's abstract in Biol. Abst. I, 11841, 1927).

In the frog ovary the degeneration of the auxocytes was the most conspicuous morphological consequence of the heat treatment. This could lead to the impression that the female differentiator depends on the presence and on certain functions of germ cells. Against such a view stands the fact mentioned above, that this degeneration is not the first effect of the high temperature, but the cessation of all further ovocyte differentiation and the beginning of the medullary growth. This suggests that the female differentiator may rather be located in some somatic cells, and we ventured the hypothesis that it is the granulosa which produces the female morphogenetic substance. This assumption receives support from two of the author's previous experiments with overripe eggs (Witschi, '24). Overripeness can change the sex of females even if it has not visibly affected the migration and the morphological structure of the primordial germ cells. In

[2] For extensive literature list see Domm, '27.

such cases the cultures consist first—in the given case at metamorphosis time—of males and females in equal numbers. Some time later, the females change into males. This seemingly spontaneous transformation must be interpreted as an after-effect, as insufficiency of either the gonial or the somatic constituent of the ovary. A further experiment decides this alternative, showing, that those gonomeres which lie in a body region specially affected by overripeness change their sex first. As the germ cells reach their sites in the gonads relatively late in the embryonic life, immigrating from an entirely different body region, this correlation must depend upon the mesodermal elements of the germinal epithelium.

Still one might object to such a conclusion for the reason that in no case the female differentiator was found active in the absence of germ cells. This is, of course, an unsettled point. In our experiments, apparently, the germ cells were more resistant than the female differentiator. However, it is not precluded that under different conditions a sterile ovary would grow with persisting ovarial sacs, inducing the growth of oviducts and the development of the physiological sex characters. For we have to remember that Brambell and his collaborators found this condition in some of their x-rayed mice.

They report that, despite the disappearance of the ovocytes, the secondary sex characters developed normally and when the animals reached the adult stage their oestrous cycles were normal and regular.

A distinctive feature of the sex reversal in frogs is the transformation of the ovarial sac into the rete and tubular apparatus of the testis. This constitutes a real inversion of a sex dimorphic structure into its opposite without elimination of any cells during this process. This differs greatly from what we occasionally called a transformation of ovogonia into spermatogonia, for, as it has been shown, all germ cells which were differentiated in a female sense disintegrate and only undifferentiated gonia become male sex cells.

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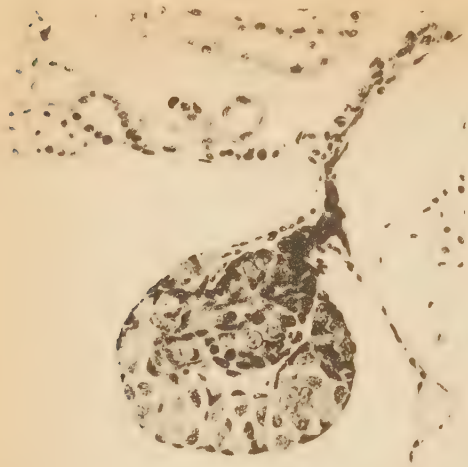

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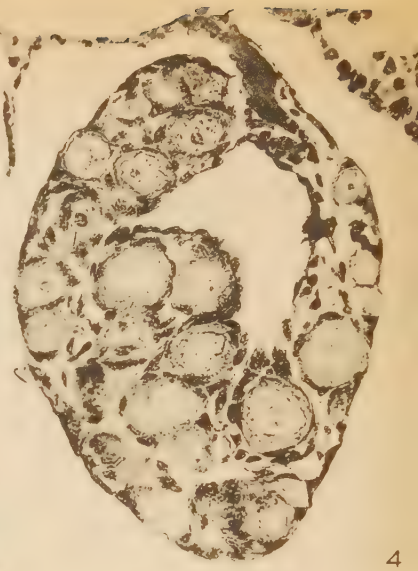

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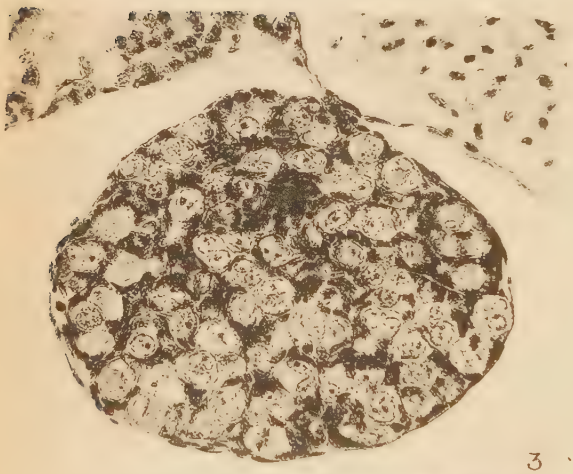

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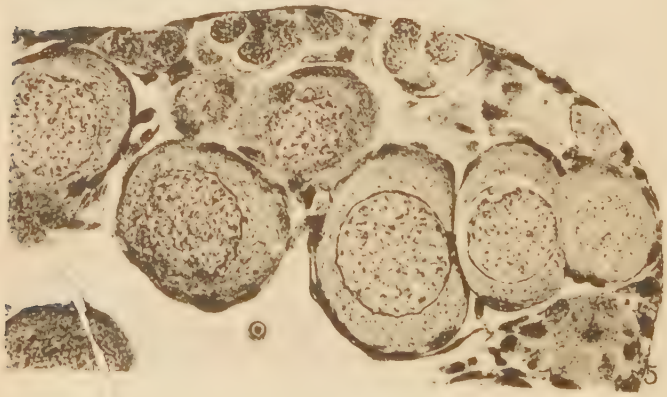

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PLATE 1

EXPLANATION OF FIGURES

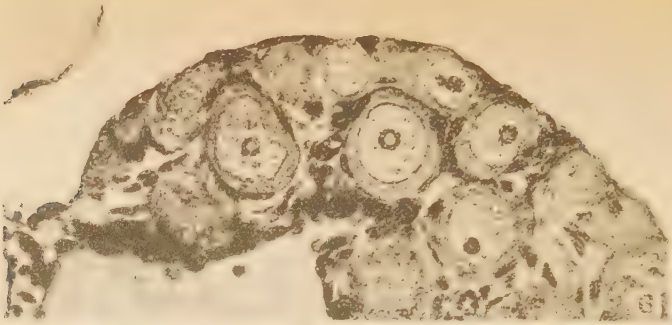
1 Rana sylvatica (no. 27), thirty-three-day-old larva. Incomplete immigration of the germ cells has resulted in the formation of a hermaphroditic gonad. Cortex with ovogonia and ovocytes (synaptene); medulla with spermatogonia. Photomicrograph, × 200.

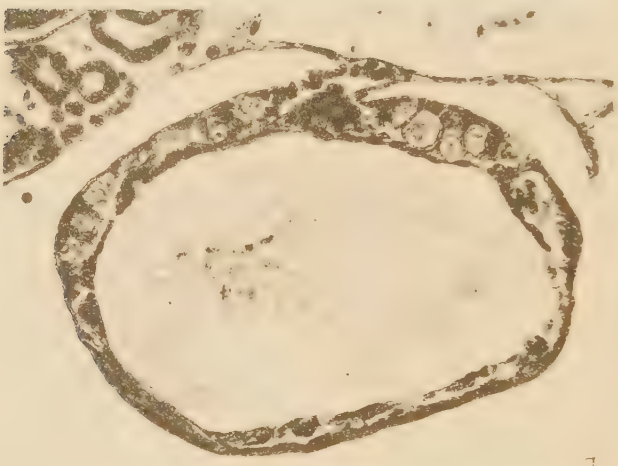
2 Rana sylvatica, sixty-seven days old. No. 52, typical male gonads at the end of the heat experiment. No. 36, the most ovary-like female gonads at the same time. The left gonad is more slender than the right one. Camera lucida, × 10.

3 Rana sylvatica, no. 52 (compare fig. 2); cross-section through right testis.

4 Rana sylvatica, no. 1, forty-nine days old. Cross-section through left ovary after fifteen days of heat treatment. Photomicrograph, × 300.

5 Rana sylvatica, normal control no. 55, forty-four days old. Part of the normal germinal epithelium. Photomicrograph, × 400.





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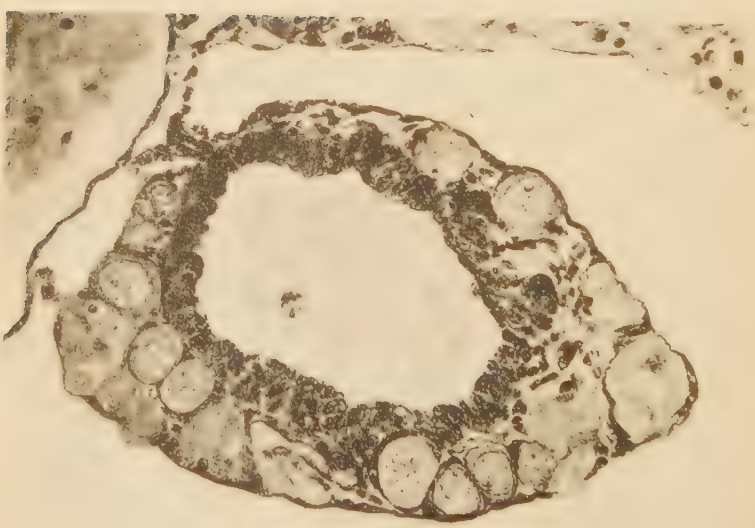

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PLATE 2

EXPLANATION OF FIGURES

6 Rana sylvatica, no. 1, forty-nine days old. Part of the germinal epithelium after fifteen days of heat treatment. Photomicrograph, × 400.

7 Rana sylvatica, no. 36 (compare fig. 2). Right ovary after thirty-three days of heat treatment. Photomicrograph, × 300.

8 Rana sylvatica, no. 4; forty-nine days old; fifteen days of heat treatment. Beginning of medullary growth. Photomicrograph, × 300.

PLATE 3

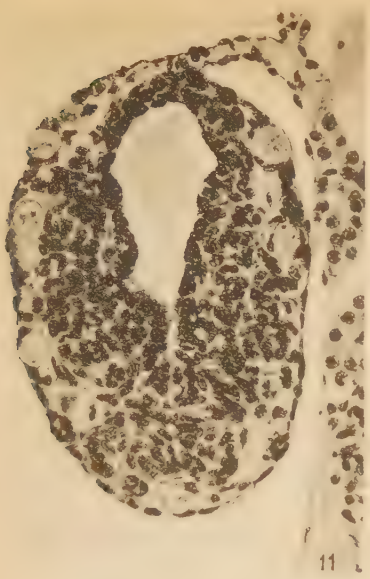
EXPLANATION OF FIGURES

9 Rana sylvatica, no. 12; fifty-one days old; seventeen days of heat treatment. Photomicrograph, × 300.

10 Rana sylvatica, no. 9; fifty-one days old; seventeen days of heat treatment. Formation of seminiferous tubules. Photomicrograph, × 300.

11 Same animal as in figure 10. Formation of rete tubules. Photomicrograph, × 300.

12 Rana sylvatica, no. 11; fifty-one days old; seventeen days of heat treatment. Photomicrograph, × 300.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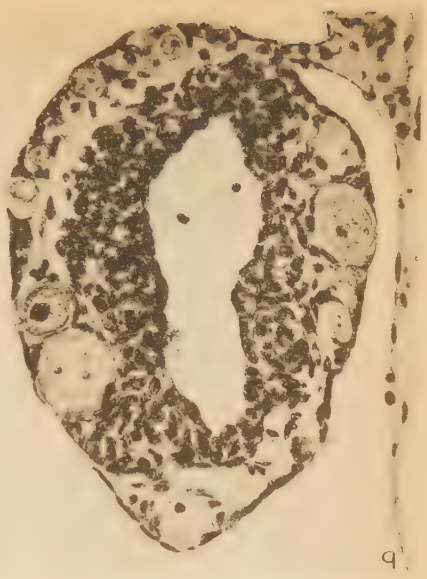
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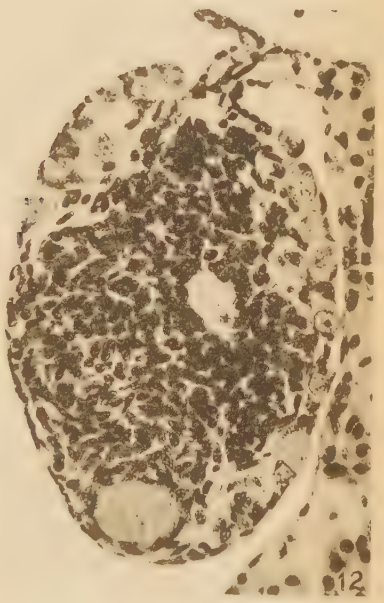
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PLATE 4

EXPLANATION OF FIGURES

13 Rana sylvatica, no. 24; sixty-three days old; twenty-nine days of heat treatment. Almost complete tubular apparatus. 'Ovogonia,' but no spermatogonia. Photomicrograph, × 300.

14 Rana sylvatica, no. 27; sixty-three days old; twenty-nine days of heat treatment. The germ cells leave the germinal epithelium. Photomicrograph, × 300.

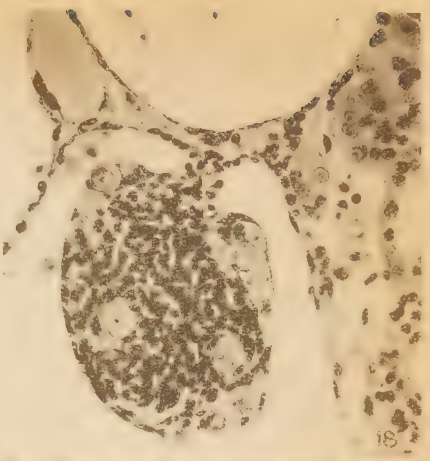
15 Rana sylvatica, no. 34; sixty-seven days old; thirty-three days of heat treatment. The germ cells migrate toward the medulla. Photomicrograph, × 300.

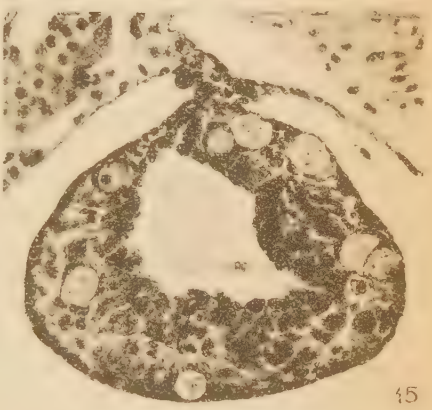
16 Same animal as in figure 14. Photomicrograph, × 300.

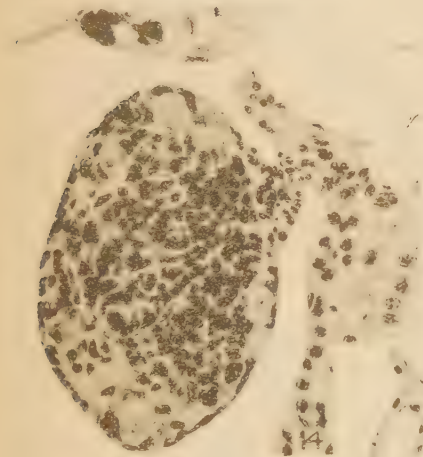
17 Rana sylvatica, no. 48; sixty-seven days old; thirty-three days of heat treatment. Migration of the germ cells. Photomicrograph, × 300.

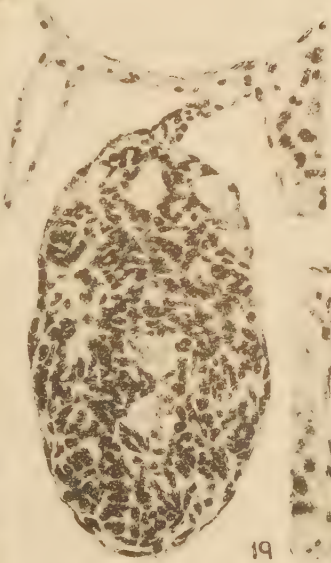
18 Rana sylvatica, no. 25; sixty-three days old; twenty-nine days of heat treatment. Some germ cells have become embedded into the medulla. Photomicrograph, × 300.

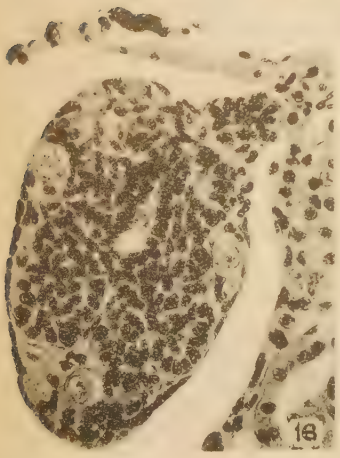
19 Same gonads as in figure 18. Photomicrograph, × 300.











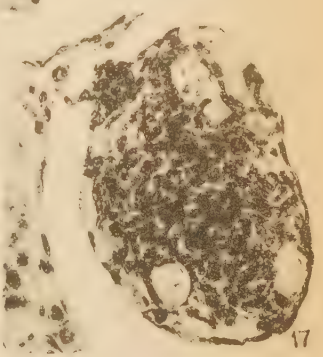


PLATE 5

EXPLANATION OF FIGURES

20 Rana sylvatica, no. 53; sixty-seven days old; thirty-three days of heat treatment. Nearly typical testis. Photomicrograph, × 300.

21 Rana sylvatica, no. 22; sixty-three days old; twenty-nine days of heat treatment. Photomicrograph, × 300.

22 Rana sylvatica, no. 18; sixty days old; twenty-six days of heat treatment. Photomicrograph, × 300.

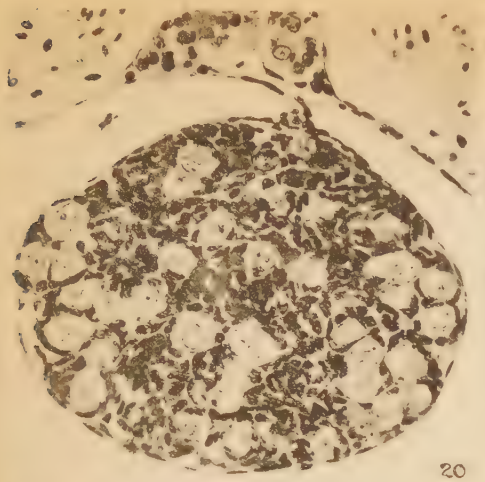
23 Same animal as in figure 22.

24 Rana sylvatica, no. 54; sixty-seven days old; thirty-three days of heat treatment. Photomicrograph, × 300.

25 Rana sylvatica, no. 28; sixty-three days old; twenty-nine days of heat treatment. Photomicrograph, × 300.

26 Rana sylvatica, no. 21; sixty days old; twenty-six days of heat treatment. Two giant spermatogonia migrate into the medulla. Photomicrograph, × 300.

27 Diagram illustrating the 'sex value' of the different regions of the gonad. The medulla is the seat of the male differentiator (*M*). The peritoneum (solid black) is indifferent, while in the deeper layers of the cortex the female differentiator (*F*) is located.



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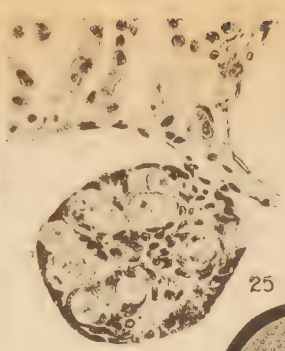

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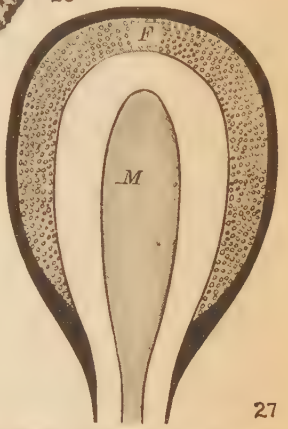

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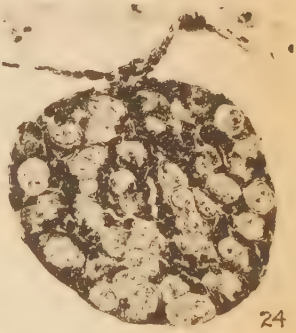

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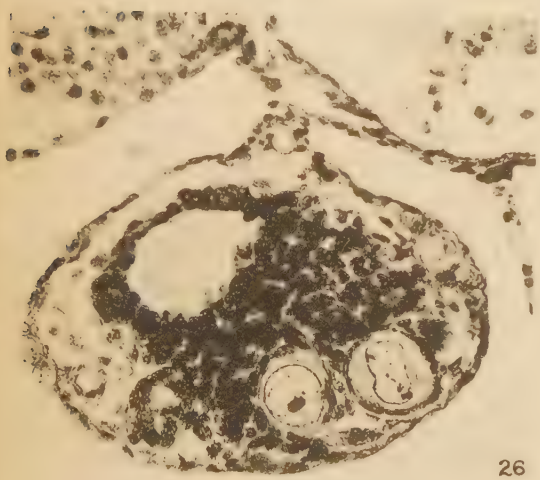

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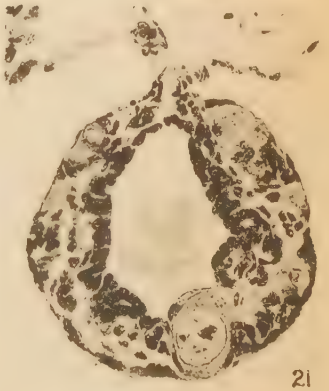

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Integumentary Pigmentation in Frog During Metamorphosis, with Especial Reference to Tail Skin Histolysis.

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Several types of larval skin transplantation experiments were made in an attempt to determine the local specificity of frog integument during metamorphosis when transplanted to foreign regions. The grafts involved skin from the back, belly, side and tail. Observations were made with respect to specificity of the integument for pigmentation and histolysis.

Autoplastic and homoplastic larval skin grafts showed local specificity for pigmentation upon larval transformation. All grafts invariably developed the pigment pattern during metamorphosis characteristic of the region from which they had been removed. Skin transplanted from back to side, or side to back, developed normal spotting characteristic of the region from which they had been taken. Skin transplanted from back to belly and vice versa, produced its normal spotting in the new position.

It is concluded that the integument of the frog *(Rana pipiens)* is specific for pigmentation and the factors responsible for the development of the normal pattern, during metamorphosis, are contained within the skin itself. Moreover, it may be concluded that certain factors within the blood stream are active or become active at a certain stage of metamorphosis and might well be conceived as being responsible for the rapid development of the latent pigmentation pattern present in the various areas of the integument.

Similar transplants involving an interchange of tail skin with skin from the back, suggested a specificity of tail skin toward histolysis. Conversely, skin transplanted from the back to the tail undergoes normal development during metamorphosis and develops the pigmentary pattern characteristic of the region from which it was taken. Instead of such grafts histolyzing and being absorbed due to their contact with atrophying tissues of the tail during metamorphosis, as might be expected, they invariably remained healthy and developed the normal pigment pattern characteristic of back skin. More-

over, the pigmentary changes of such grafts were found to take place simultaneously with the spotting of the back integument. Histological examination showed no signs of cellular histolysis, the integument having the appearance of normal back skin in every respect.

The reciprocal graft, however, in which tail skin was transplanted to the back underwent pronounced degeneration during metamorphosis and presented all of the typical macroscopic signs of histolysis. As metamorphosis progressed, the grafts were resorbed simultaneously with the resorption of the integument of the tail. Histological examination revealed cellular histolysis identical to that typical of histolyzing tail integument during the atrophy of the tail.

The results of the present work serve to throw light on the possible mechanism of tail skin histolysis as it occurs during normal atrophy of the tail during metamorphosis. It has been suggested by Barfurth[1] and Bataillon[2] that atrophy of the tail is induced primarily by the rapid growth of the urostyle during metamorphosis, which serves at least partially to occlude the blood supply to the tail. This, according to Bataillon[2] and Morse[3] would be a possible explanation for the condition of acidosis which occurs in the tissues of the atrophying tail. Morse, furthermore, points out that the acidosis present is sufficient to bring about autolytic reactions resulting in the atrophy of the tail. More recently, however, Helff[4] has shown that the urostyle cannot be the fundamental causative factor involved since typical tail atrophy was found to occur in animals in which the anlagen of the urostyle had been extirpated prior to metamorphosis.

The results of the present work tend to support Helff's contention. The fact that tail integument transplanted to the back undergoes normal histolysis without the presence of adjoining atrophying tissue certainly requires some other explanation. Moreover, the fact that integument from the back transplanted to the tail never undergoes histolysis during tail atrophy is a negation of the idea that tail skin undergoes histolysis during metamorphosis due entirely to its association with the atrophying musculature beneath. It is concluded that 2 factors probably determine the histolysis of tail integument, one of which lies within the skin itself and is specific, and the second, within the blood stream, which becomes functional in inducing histolysis of tail integument at a certain stage of metamorphosis.

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1 Barfurth, D., *Arch. f. miki. Anat.*, 1887, xxix, 1.

2 Bataillon, E., *Ann. de Universite de Lyons*, 1891, II.

3 Morse, W., *Biol. Bull.*, 1918, xxxiv, 149.

4 Helff, O. M., *Anat. Rec.*, 1928, Dec., 39.

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Methoden der Vererbungsforschung bei Tieren

Von Emil Witschi, Iowa

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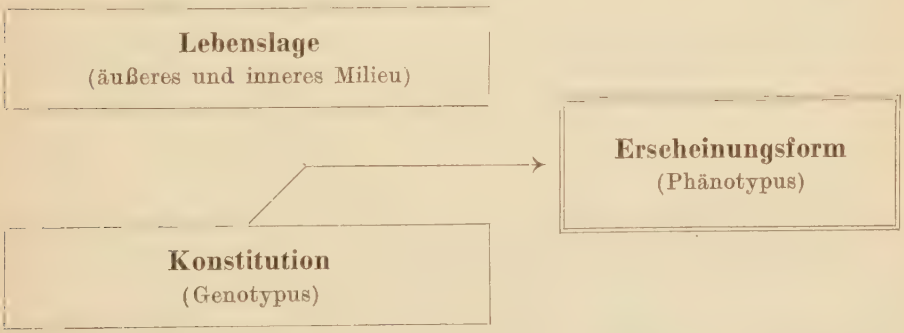
ALLGEMEINER TEIL.

Von **Emil Witschi**, Iowa.

(Mit 18 Abbildungen.)

I. Die Variabilität, ihre Bedeutung und biometrische Behandlung.

Die Zeit ist nicht fern, da man glaubte, mittels der Massenstatistik die Vererbungsgesetze erfassen zu können. Heute hat die Biometrik in der Vererbungsforschung nur noch die Bedeutung einer Hilfswissenschaft. Ihre Anwendung wird notwendig auf Grund der Tatsache, daß die Erscheinungsform (Phänotypus) eines Organismus — als Ganzes sowohl als auch bezüglich einzelner Eigenschaften — nicht nur durch die ererbte Konstitution (Genotypus), sondern auch durch die Lebenslage bestimmt wird. Die Erscheinungsform ist die Resultante aus zwei Faktorengruppen:



Wir können uns an dieser Stelle einer eingehenden Behandlung der biometrischen Methoden und namentlich der Ableitung der gebräuchlichen Formeln entschlagen, da dieselben bereits durch *V. Haecker* in diesem Handbuch (Abt. IX, Teil 3, H. 1, S. 93 bis 138) eine entsprechende Darstellung erfahren haben. Hier interessiert vorwiegend die Frage, wie die Störungen, welche von den m i l i e u-

bedingten Variationen (heute oft als Modifikationen bezeichnet) in die Vererbungsexperimente hereingetragen werden können, zu eliminieren bzw. in Rechnung zu stellen sind.

Eine durchweg verbindliche Regel ist nicht zu geben, da sich die Merkmale als ungleich variabel erweisen. Es ist darum notwendig, sich bei jeder Untersuchung zu vergewissern, in welchen Grenzen und auf Grund welcher Faktoren die verfolgten Eigenschaften abändern. Die Ernährungsweise wird relativ am häufigsten einen Einfluß geltend machen. Die Beziehungen zwischen Nahrungsmenge und Körpergröße sind bekannt. In der Regel wird man den Versuchstieren eine passend zusammengesetzte und eventuell variierte Nahrung bis zum Überfluß zur Verfügung stellen. Die qualitative Zusammensetzung spielt oft eine wesentliche Rolle. *Pictet* hat gezeigt, daß die Färbung der Schmetterlinge durch das den Raupen gebotene Futter beeinflußt werden kann. Extreme Wirkungen kommen durch Hormone, Vitamine, einzelne Metallionen usw. zustande, die, in variierten Dosierungen verabreicht, Wachstum und Differenzierung in weitem Maße beeinflussen können.

Die Entwicklung aller wechselwarmen Tiere ist in hohem Grade von der Temperatur abhängig. In der optimalen Zone wird sie gewöhnlich entsprechend dem *van 't Hoff*schen Koeffizienten beschleunigt. Unter extremen Bedingungen verläuft sie dagegen langsamer, d. h. der Koeffizient Q_{10} steigt in der „Kälte" über drei und sinkt in der „Hitze" unter 2. Für die Larvenentwicklung des Grasfrosches (R. temporaria) wurden z. B. folgende Werte erhalten:

TABELLE 1.

Temperaturintervall (Celsiusgrade)	Temperaturkoeffizient Q_{10}
10—15	6·06
15—21	2·65
21—27	1·37

Die einzelnen Komponenten der Entwicklungsprozesse werden indessen nicht gleichmäßig beschleunigt bzw. verzögert. *Doms* (1915) hat für eine Reihe somatischer Organe des Frosches nachweisen können, daß sie sich bei verschiedenen Temperaturen qualitativ ungleich (disproportional) entwickeln. Besonders auffällig ist der Effekt auf die Keimdrüsen. Während z. B. die alpinen Rassen bei 21° glatt das mechanische Zahlenverhältnis der Geschlechter von 1 : 1 ergeben, entwickeln sich bei 10° die Männchen und bei 27° die Weibchen als protogyne Hermaphroditen. Auf diese Weise kann auf bestimmten Entwicklungsstadien das erblich fixierte Zahlenverhältnis durch Lebenslagefaktoren verwischt werden.

Zahlreiche weitere Faktoren, wie Feuchtigkeit und Licht können ebenso die Ausprägung von Erbeigenschaften vollkommen verändern. *Morgan* und *Bridges* (1916) berichten, daß das Merkmal „abnormales Abdomen" der Drosophilafliege nur in feuchten Kulturen zur Ausbildung kommt, während bei Trockenheit die gleichkonstituierten Fliegen normal erscheinen. Der Einfluß des Lichtes auf die Ausfärbung vieler Fische ist bekannt und muß bei Vererbungsstudien über Farbmerkmale in Rechnung gestellt werden.

Aus alledem ergibt sich die Forderung, daß bei Vererbungsexperimenten die miteinander zu vergleichenden Tiere unter möglichst identischen Kulturbedingungen herangezüchtet werden sollen. *Evans Burr* und *Goettsch* (1927) haben gezeigt, daß hochgradig ingezüchtete und infolgedessen genetisch kaum mehr sehr verschieden konstituierte Ratten bei Fütterung mit chemisch gereinigten und sorgfältig kontrollierten Nahrungsgemischen immer noch eine beträchtliche Variabilität der Wachstumskurven zeigen. Diese heute noch unerklärte Restvariabilität macht das Arbeiten mit größeren Gruppen und die Anwendung der Fehlermethoden bei der Darstellung der Resultate unerläßlich.

In Betracht zu ziehen sind dann immer noch mögliche jahreszeitliche Variationen, Wirkungen hervorgerufen durch funktionelle Anpassung und eventuelle Veränderungen, welche die Eigenschaften mit dem Alter der Vergleichstiere erfahren.

Wo eine größere Variabilität nicht vermieden werden kann, wird man gegebenenfalls die Mittelwerte bestimmen und mit diesen arbeiten. Das ist fast immer notwendig, wenn quantitative Merkmale, wie Körpergröße, Milchertrag, Aschegehalt u. ä., in ihrem Vererbungsmodus verfolgt werden sollen. Die Berechnung erfolgt nach der Formel[1]):

$$\text{Mittelwert } M = \frac{\Sigma pV}{n}$$

Dabei bedeuten:
Σ = Summationszeichen.
p = Anzahl der Individuen einer Variantenklasse.
V = Absolutes Maß der Variante.
n = Gesamtzahl der Individuen.

Sein mittlerer Fehler beträgt:

$$m = \pm \frac{\sigma}{\sqrt{n}}$$

σ = Standardabweichung.

[1]) *Haecker*: l. c. S. 101/105.

Häufig ist es von Vorteil, auch das Maß der Variabilität zu bestimmen. Dazu wird heute mit Vorliebe der aus der Standardabweichung in Prozenten des Mittelwertes errechnete Variationskoeffizient (v) verwendet:

$$v = \frac{100\,\sigma}{M}.$$

Es hat sich immer wieder bestätigt, daß die Variabilität unter optimalen Bedingungen am geringsten ist. Im oben erwähnten Kaulquappenversuch wurden z. B. folgende Werte ermittelt:

TABELLE 2.

Temperatur Cels.	v
10°	4·36
15°	3·54
21°	3·21
27°	5·64

Falls nicht besondere Umstände anderes verlangen, gilt daher auch als Regel, daß die Zuchten unter optimalen Kulturbedingungen zu führen sind.

Die einem einzelnen Erbfaktor (*Gen*) entsprechenden phänotypischen Realisierungsmöglichkeiten hat man nach *Woltereck* als seine Reaktionsnorm bezeichnet. Diese Bezeichnung ist meines Erachtens durchaus nicht glücklich, da die ganze Variationsbreite und nicht lediglich die Norm inbegriffen sein soll. Der Ausdruck „Realisierungsmöglichkeiten" scheint mir darum vorteilhafter. Die beiden aufgestellten Regeln der identischen und der optimalen Kulturbedingungen verfolgen beide das Ziel, die Variationsbreite der tatsächlichen Realisierungen möglichst einzuschränken.

Die statistische Behandlung der Variabilität kann selbstredend die biologische Analyse nur unterstützen, nie ersetzen. Mathematisch exakte Daten, die aus Materialien von unbekannter Lebenslage errechnet wurden, bleiben für den Vererbungsforscher häufig wertlos, wenn nicht gar irreführend.

II. Die Analyse der in Chromosomen lokalisierten Erbfaktoren (Mendelismus).

Die Möglichkeiten der vorwiegend geübten Bastardierungsmethoden beruhen auf dem Kernphasenwechsel, der sich bei normaler geschlechtlicher Fortpflanzung zweimal im Verlauf einer Generation vollzieht. Mit wenigen Ausnahmen repräsentieren

die Tiere in ihrer charakteristischen Form den diploiden oder zygotischen Zustand. Bei der Gametenbildung vollzieht sich die Reduktion auf die haploide oder gametische Phase. Durch die Vereinigung von Ei und Samenfaden wird bei der Befruchtung die Diplophase wieder hergestellt (vgl. Fig. 52).

Ausnahmsweise — z. B. bei Amoeba diploidea — ist die Diplophase durch Doppelkernigkeit ausgezeichnet. In der Regel verschmelzen jedoch die väterlichen und mütterlichen Kerne bald nach der Befruchtung. Das allgemeinste Kennzeichen der Diplophase ist das Vorhandensein doppelter Chromosomensortimente in den Zellkernen. Der Reduktionsprozeß bewirkt die Sonderung von zwei kompletten einfachen Sortimenten, welche indessen nur bei Homozygoten vollkommen gleichwertig ausfallen können. Die Fig. 53 zeigt in schematisch

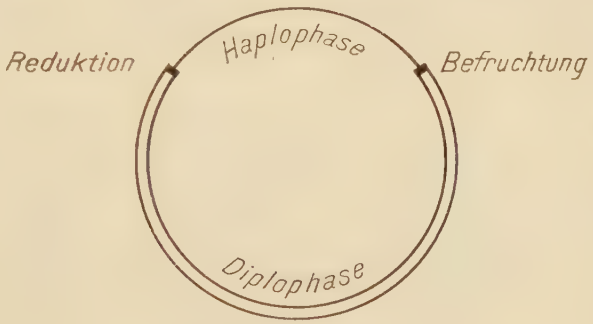


Fig. 52. Schema des Kernphasenwechsels.

vereinfachter Darstellung die Hauptphasen des Reduktionsvorganges. Es wurde die einfache Annahme von nur zwei Chromosomenpaaren zugrunde gelegt. In der Regel sind es mehr, doch wird die Zahl 40 nur selten überschritten.

Der Vererbungsmodus der „mendelnden" Erbfaktoren oder Gene wird durch das Verhalten der Chromosomen bestimmt. Drei Tatsachenkomplexe sind besonders bedeutungsvoll.

1. Am Ende der Diplophase konjugieren die Chromosomen paarweise miteinander. Die mikroskopischen Untersuchungen geben bisher noch nicht mit befriedigender Klarheit Auskunft über die dabei sich abspielenden Vorgänge. Doch lassen die Bastardierungsergebnisse keinen Zweifel darüber bestehen, daß die homologen Teile der Chromosomen (die man etwa homologe Chromomeren nennen mag) nicht verschmelzen, sondern ihre Selbständigkeit bewahren. Damit bleibt auch die Integrität der Erbfaktoren gewahrt, und die Reduktionsteilung bewirkt eine reine Scheidung väterlicher

und mütterlicher Gene. Dieses „Spaltungsgesetz" ist schon von *Mendel* (1865) aus der Gametenreinheit erschlossen worden[1]).

2. Beim Aussondern haploider Garnituren im Reduktionsprozeß kombinieren sich mütterliche und väterliche Chromosomen in freier Weise (Fig. 54). Entsprechend herrscht auch freie Kombination zwischen den in verschiedenen Chromosomen[2]) liegenden Genen. Diese freie, den Regeln der Wahrscheinlichkeitsrechnung folgende Kombinationsmöglichkeit nachgewiesen zu haben, ist ein weiteres Verdienst *Mendels* (1865). Demgegenüber sind Gene, welche im gleichen Chromosom liegen, gekoppelt. Nachdem bereits verschiedene Autoren (*Bateson*, *Punnett*, *Baur*)

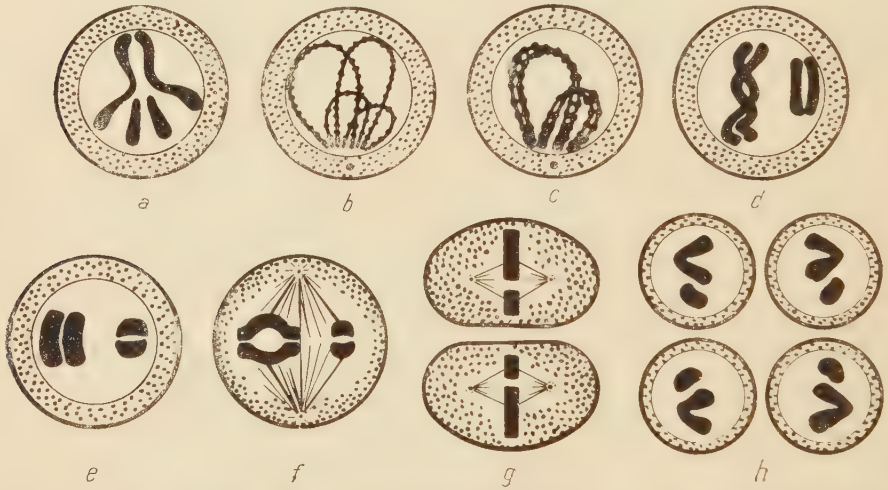


Fig. 53. Die Stadien der Chromosomenreduktion bei der Keimzellreifung. *a* = Diploider Chromosomensatz einer Keimzelle im Vermehrungsstadium; *b* bis *d* = Chromosomenconjugation während der Proreduktion; *b* = frühes Synapsisstadium; *c* = spätes Synapsisstadium; *d* = Strepsinemastadium; *e* bis *h* = Reifeteilungen; *e* = Diakinese; *f* = erste Reifeteilung (hier als Reduktionsteilung dargestellt); *g* = zweite Reifeteilung (hier Aequationsteilung); *h* = Gameten mit haploiden Chromosomensätzen.

bei botanischen Vererbungsstudien mit der Erscheinung bekannt geworden waren, konnte *Morgan* (1910, 1911) auf Grund ausgedehnter Arbeiten mit der Drosophilafliege die Faktorenkopplung (Linkage) auf die gemeinsame Lokalisation im selben Chromosom zurückführen.

[1]) In der Literatur finden sich mehrfach Angaben über „Gametenunreinheit". Es liegt kein Grund vor, die Möglichkeit eines Substanzaustausches zwischen Chromomeren und damit einer Vermischung von Erbfaktoren von vornherein abzustreiten. Dagegen ist ein solcher Vorgang bis heute doch noch nie mit überzeugender Klarheit nachgewiesen worden.

[2]) Auf den diploiden Zustand bezogen, müßten wir sagen ... verschiedenen Chromosomen paaren ...

3. Die einzelnen Chromosomen bewahren zwar weitgehend ihre Individualität. Doch können gelegentlich zwischen homologen Chromosomen (d. h. zwischen dem väterlichen und dem mütter-

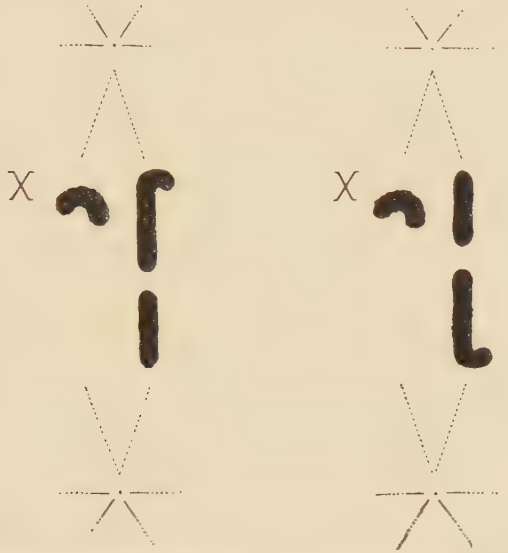


Fig. 54. Kombinationsweisen des Geschlechtschromosoms (*X*) und eines Autosomenpaares in den Reduktionsteilungen im Hoden einer Heuschrecke. Das *X*-Chromosom ist mütterlicher Herkunft. Es kombiniert sich eben so oft mit dem väterlichen Chromosom des Autosomenpaares als mit dem mütterlichen. Die beiden zeigen hier eine konstante morphologische Differenz: terminale bzw. subterminale Anheftung der Zugfaser. (Nach *Carothers* aus *Morgan*.)

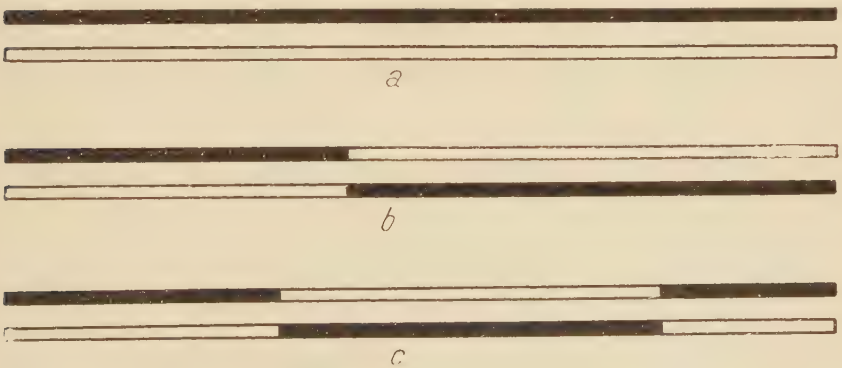


Fig. 55. Schematische Darstellung des Austauschphänomens.
a = Chromosomenpaar vor dem Austausch; *b* = nach einfachem Austausch; *c* = nach doppeltem Austausch.

lichen Partner eines Chromosomenpaares) entsprechende Stücke ausgetauscht werden (Fig. 55). Dieser von *T. H. Morgan* (1911) aus Bastardierungsresultaten erschlossene und als Crossing over

bezeichnete Vorgang scheint sich nur während der Keimzellreifung, ungefähr im Synapsis-Strepsinema-Stadium zu vollziehen und ist bei Drosophila auf das weibliche Geschlecht beschränkt. Bei anderen Tieren (z. B. Kaninchen, Castle 1924, Fischen, Aida 1921) wurde er auch in der männlichen Gametenbildung nachgewiesen. Der Austausch chromosomaler Teile hat die Neukombination gekoppelter Gene (recombination of linked charakters, Morgan 1923) zur Folge.

Neben den aus dem normalen Kernphasenwechsel sich ergebenden Möglichkeiten haben in den letzten Jahren neue Methoden der Vererbungsforschung immer mehr Bedeutung erlangt, welche sich auf die Analyse von Pflanzen und Tieren mit ungewöhnlichen Chromosomenkombinationen beziehen.

A. Zuchtmaterial und Zuchtmethoden.

Mendel arbeitete sowohl mit Pflanzen als mit Tieren, konnte jedoch nur mit ersteren Resultate von Bedeutung gewinnen. Auch die neue Ära der Vererbungsforschung setzte mit botanischen Arbeiten ein (*Correns*, *de Vries*, *Tschermak*), und solche behielten einen weiten Vorsprung durch ein ganzes Jahrzehnt hindurch. Die Gründe sind leicht ersichtlich in den einfachen Kulturbedingungen (1) und der großen Vermehrungsfähigkeit (2) der Pflanzen gegeben. Von höheren Tieren vermögen weder Hühner noch die verschiedenen Nagerspezies in diesen beiden Punkten mit den Blütenpflanzen in Konkurrenz zu treten. Dagegen besitzen manche Evertebraten eine starke Vermehrungsrate, und rasche Generationenfolgen. Schon *Mendel* begann Versuche mit Bienen. Das erste große zoologische Material haben *Coutagne*, *Kellogg* und *Toyama* mit ihren Seidenspinnerzuchten beigebracht.

Aus der Tatsache, daß Kulturpflanzen und Haustiere zuerst mit Erfolg benutzt werden konnten, geht hervor, welch große Bedeutung der züchterischen Erfahrung (3) in der Vererbungsforschung zukommt. Es lag nahe, hier auch die Fertigkeit der entomologischen Liebhaber der Wissenschaft dienstbar zu machen. Der Erfolg entsprach nur teilweise den gehegten Erwartungen, weil gerade die meist gepflegten Tagfalter schwer über mehrere Generationen hinweg zu züchten sind. (Vgl. *E. Fischer*, dieses Handbuch, Abt. IX, Teil 1.) Dagegen konnte *Goldschmidt* mit relativ geringer Mühe den Schwammspinner (Lymantria dispar) durch viele Jahre hindurch züchten und so ein riesiges Material gewinnen. Durch die Notwendigkeit, die Tiere in engen Behältern unter strenger Abgeschlossenheit gegenüber ihresgleichen zu halten, wird mancher interessante Versuch entweder

vereitelt oder doch seine Ausdehnung zwecks Gewinnung größerer Zahlen verunmöglicht. Viele Arten, selbst Haustiere, wie z. B. Hunde, zeigen nämlich unter solchen Bedingungen eine herabgesetzte Fruchtbarkeit oder gar ein völliges Schwinden der Propagation.

Des weiteren wird die Eignung des Materials bestimmt durch das Vorhandensein von Mutationen (4) bzw. durch die Häufigkeit mit der solche auftreten. Als Mutation im weiteren Sinne bezeichnen wir jede Erbvariation. Für Bastardierungsexperimente am besten geeignet sind solche, welche den folgenden Anforderungen genügen: 1. Leichte Kenntlichkeit, so daß die Unterscheidung vom Gegencharakter keine Mühe macht. 2. Beständigkeit gegenüber Milieueinflüssen. 3. Wahrung der vollen Lebenstüchtigkeit aller Keime und der verschiedenen Nachkommenkombinationen. 4. Klare und konstante Dominanz. Ein intermediäres Verhalten der Bastarde ist oft vorteilhaft, weil es die Sonderung der Homozygoten erleichtert. Bei Polyhybridismus werden dann allerdings die Zahlenverhältnisse bedeutend kompliziert.

Nur wenn diese Bedingungen erfüllt sind, darf man reine Zahlenverhältnisse in den Versuchsresultaten erwarten. Selbstverständlich wird das Interesse des Forschers sich oft solchen Mutationen zuwenden, welche diesen Forderungen nicht in allen Punkten entsprechen. Abweichungen von den Spaltungszahlen bedürfen dann in der Regel einer biologischen — nicht einer mathematischen — Interpretation.

Schließlich wird häufig die praktische oder theoretische Bedeutung (5) für die Wahl der Versuchsobjekte von Bedeutung sein. Die Arbeit mit Haustieren ist namentlich in landwirtschaftlichen Instituten gepflegt worden. Über die besonderen dabei ausgearbeiteten Methoden wird im nachfolgenden Abschnitt (*H. Nachtsheim*: Säugetiere) berichtet.

In theoretischer Hinsicht hat in den letzten anderthalb Dezennien das Werk von *T. H. Morgan* und seiner Mitarbeiter eine überragende Bedeutung gewonnen. Die Fruchtfliege Drosophila melanogaster ist nach dem Stande unserer heutigen Kenntnisse als das ideale Objekt der Vererbungsforschung zu bezeichnen, das in seiner Eignung sogar alle bisher verwendeten Pflanzen übertrifft. Da die Drosophila nun auch die Einführung von Laboratoriumskursen in Vererbungswissenschaft ermöglicht und immer mehr Aufnahme in Universitätsinstituten und höheren Schulen findet, so sollen hier die notwendigen Daten über ihre Biologie und Haltung zusammengefaßt werden. (Betreffend Zuchtmethoden vgl. im übrigen dieses Handbuch, Abt. IX, Teil I.)

Biologie und Zucht von Drosophila.

Die Fruchtfliege ist heute nahezu über die ganze Welt hin verbreitet. Zweifellos verdankt sie diese Universalität dem modernen ausgedehnten Fruchthandel. Ursprünglich bewohnte sie wohl nur die warmen Länder und wird auch heute noch von dort sommers nach nördlichen Strichen importiert, wo sie den Winter nicht zu überdauern vermag. Über 200 Spezies derselben Gattung sind beschrieben worden, von denen namentlich D. simulans der melanogaster sehr ähnlich sieht. Diese beiden Spezies sind auch die einzigen, welche bis jetzt gekreuzt werden konnten. Die Bastarde waren ausnahmslos steril. Mutationen sind bis 1925 bei 13 Spezies beschrieben worden.

Wie aus der folgenden Tabelle ersichtlich, weitaus am häufigsten (zirka 400) bei Melanogaster; in weiteren vier Arten sind es ungefähr je 40, und von acht Arten kennen wir nur vereinzelte Gene.

TABELLE 3.

Übersicht der Drosophilaspezies mit beschriebenen Mutationen nach dem Stande von 1925.

Spezies	Haploide Chromosomenzahl	Zahl der Koppelungsgruppen	Zahl der geschlechtsgebundenen Gene	Zahl der autosomalen Gene	Körperlänge *mm*	Körperfarbe
Melanogaster . . .	4	4	150	250	2	gelb
Simulans	4	3	15	25	2	„
Willistoni	3	3	30	9	2	„
Obscura	5	5	28	12	2·2	schwarz
Virilis	6	6	18	23	2·5	„
Busckii	4	2	0	3	2	gelb
Immigrans	4	3	1	5	2·5	„
Similis	6	1	0	1	2·2	„
Funebris	6	3	3	4	2·5	braun
Hydei	6	2	2	2	2·5	grau
Repleta	6	2	2	1	2·5	„
Affinis	5	1	0	2	2·2	schwärzlich
Caribbea	4	1	0	1	2	gelb

Für Kurszwecke wird man nahezu ausschließlich D. melanogaster verwenden. Ihre wichtigeren, d. h. mit besonderem Vorteil zu benutzenden Mutationen sind aus den Chromosomenkarten der

TABELLE 4.

Die wichtigeren Mutationen der Drosophila melanogaster, die dominanten mit großen Initialen gekennzeichnet.

I		II		III		IV	
yellow	(gelb)	Star	(sternförmig)	roughoid	(rauhlich)	bent	(gebogen)
Hairy-wing	(Haarflügel)	aristaless	(aristalos)	divergent	(auseinander-weichend)	shaven	(rasiert)
scute	(schildförmig)	expanded	(ausgebreitet)			eyeless	(augenlos)
lethal	(letal)	Gull	(Mövenflügel)	sepia	(sepia)		
broad	(breit)	Truncate	(Verstümmelt)	hairy	(behaart)		
prune	(pflaumenfarbig)	dachsous	(dachsbeinig)	rose	(rosig)		
white	(weiß)	streak	(streifig)	cream III	(creme III)		
facet	(unregelmäßige Facetten)	dachs	(kurzfüßig)	Minute-h	(Klein-h)		
		Ski-II	(Ski-II)	tilt	(gekippt)		
Notch	(Gekerbt)	Jammed	(Eingekeilt)	Dichaete	(gespreizt)		
Abnormal	(Abnorm)	Minute-e	(Klein-e)	thread	(faden)		
echinus	(stachelig)	black	(schwarz)	scarlet	(scharlachrot)		
bifid	(zweigespalten)	jaunty	(aufgebogene Flügel)	warped	(abweichend)		
ruby	(rubinrot)			ski-III	(ski-III)		
crossv'less	(queraderlos)	purple	(purpur)	Deformed	(Deformiert)		
club	(klumpflügel)	cinnabar	(zinnober)	pink	(rosa)		
deltex	(deltex)	safranin	(safrangelb)	maroon	(kastanienbraun)		
cut	(abgeschnitten)	pink-wing	(rosaflügel)	dwarf	(zwergig)		
singed	(versengt)	vestigial	(stummelflüglig)	curled	(gekräuselt)		
tan	(gelbbraun)	telescope	(ausgezogen)	Hairy-wing-supressor	(Haarflügel-unterdrücker)		
lozenge	(rautenförmig)	Lobe	(Kleinäugig)				
vermilion	(zinnoberrot)	gap	(Spalte, Lücke)	Stubble	(Stoppel)		
miniature	(miniatur)	curved	(gekrümmt)	spineless	(borstenlos)		
duscky	(düster)	fringed	(gerändert)	bithorax	(doppelthorax)		
furrowed	(gefurcht)	humpy	(buckelig)	bithorax-b	(doppelthorax-b)		
sable	(samtschwarz)	arc	(gebogen)	stripe	(gestreift)		
garnet	(granat)	plexus	(wirradrig)	glass	(gläsern)		
small-wing	(kleiner Flügel)	lethal-II a	(letal-II a)	Delta	(Delta)		
rudimentary	(rudimentär)	brown	(braun)	Hairless	(Unbehaart)		
forked	(gegabelt)	blistered	(blasig)	ebony	(ebenholz)		
Bar	(Bandäugig)	purploid	(purpurfarben)	band	(band)		
small-eye	(kleinäugig)	morula	(morulaäugig)	cardinal	(hochrot)		
fused	(verschmolzen)	speck	(fleckig)	white-ocelli	(weiß-augenfleckig)		
Beadex	(Perlförmig)	balloon	(ballonförmig)	rough	(roh, rauh)		
Minute-n	(Klein-n)			crumpled	(zerknittert)		
cleft	(gespalten)			Beaded	(Glasperlenartig)		
bobbed	(gestutzt)			Pointed	(Spitz)		
				claret	(weinrot)		
				Minute	(Klein)		
				Minute-g	(Klein-g)		

Fig. 56[1]) zu ersehen. Für die Forschung kommen dagegen auch die anderen Spezies immer mehr in Gebrauch, namentlich weil aus dem Vergleich der Chromosomenkarten interessante Aufschlüsse über Konstitution und verwandtschaftliche Beziehungen zu erwarten sind. Im folgenden beziehen sich unsere Ausführungen, wo nichts Besonderes bemerkt wird, stets auf Melanogaster. (Siehe Tabelle 4.)

Die Beschaffung von Material, d. h. von Wildform-Drosophilae, und geeigneten Mutanten fällt heute nicht schwer, da solche von den verschiedenen mit ihrer Zucht beschäftigten Instituten meist gern abgegeben werden.

Für die Zucht der Fliegen werden weithalsige Pulverflaschen von 30 bis 300 cm^3 verwendet. Man kann mit Vorteil zwei verschiedene Größen wählen, die eine für Massenkulturen (Stammzuchten, Ramschkulturen), die andere für die Zucht isolierter Paare. Für Kurszwecke genügen 50 cm^3. Zum Isolieren einzelner Puppen werden kleine (5 cm^3) Glastuben verwendet. Alle Gefäße werden mit Wattebausch verschlossen. In einzelnen Instituten werden sowohl Gefäße als auch alle Instrumente vor Gebrauch sterilisiert. Größte Sorgfalt ist namentlich beim Reinigen gebrauchter Gläser zu empfehlen. Wattepfropfen, die mit Vorteil in ein weitmaschiges Gewebe eingebunden werden, können nach Heißluftsterilisation immer wieder verwendet werden. Vor Gebrauch dürfen Flaschen nicht offen stehen, weil sie sonst von Ausreißern und Wildformen als Brutplätze benutzt werden können. Ferner muß alles getan werden, um Verschimmelung zu verhüten. Da die Infektion meistens durch die Elterntiere erfolgt, ist es günstig, die Paare erst in die Zuchtgefäße zu bringen, wenn die Weibchen legereif sind, d. i. ungefähr ein Tag nach der Kopulation. Vorher werden sie am besten in kleinen Tuben mit saurem Futter gehalten.

Wenn so verfahren wird, vermögen die Larven in der Regel den Schimmel darniederzuhalten, wahrscheinlich indem sie die Sporen verzehren. Gelegentlich treten auch Bakterien oder Milben als lästige Schädlinge auf. Infizierte Kulturen werden am besten eliminiert, wenn nicht besonders wertvolle Mutanten sich darin befinden. Andernfalls kann die Linie oft durch mehrmaliges rasches Umsetzen in neue Gläser gereinigt werden.

Als Futter dient hauptsächlich Hefe, die in der Regel auf fermentierenden Früchten kultiviert und dargeboten wird. Neuer-

[1]) Namentlich in der populären Literatur werden oft mehr oder weniger passende deutsche Bezeichnungen für die Mutationen verwendet. Die Tabelle 4 enthält solche Verdeutschungen der in Fig. 56 enthaltenen Namen.

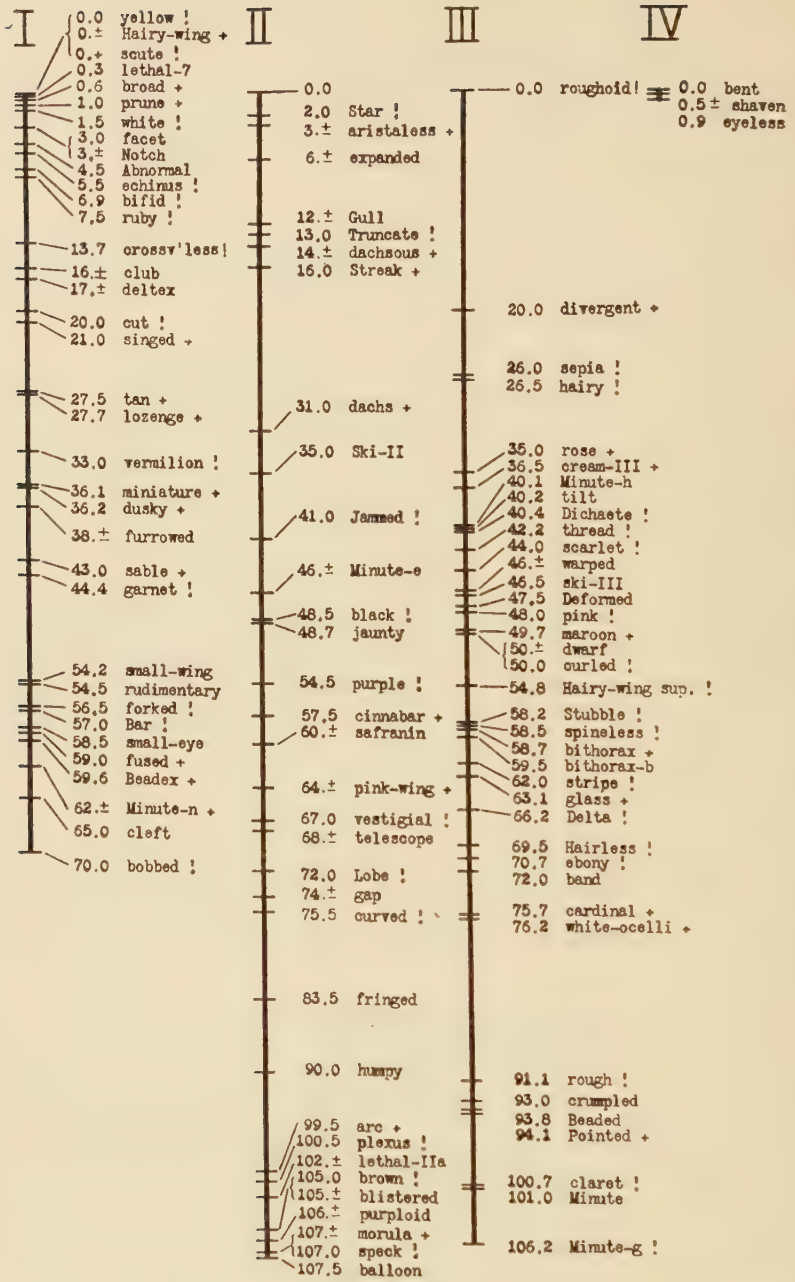


Fig. 56. Die vier Chromosomenkarten von Drosophila melanogaster. (Nach *Morgan*, 1926.)

dings werden Versuche mit Molassenährböden gemacht[1]). *Castle* verwendet rohe, in Scheiben geschnittene Bananen, *Babcock* und *Collins* sterilisieren diese durch längeres Kochen, und im Laboratorium von *Morgan* wird eine Bananen-Agar-Mischung gekocht. Als besonderer Vorzug der Bananennährböden wird die Resistenz gegen Verschimmelung hervorgehoben. In Europa wird man dem Rezept von *Just* den Vorzug geben, das in Anlehnung an *Morgan* ausgeführt ist, jedoch die teuren Bananen durch Pflaumen ersetzt: 1 Pfund getrockneter Pflaumen wird weich gekocht, abgekühlt, entkernt, zerkleinert und nochmals längere Zeit gekocht. In den heißen Brei wird eine Lösung von 10 *g* Agar-Agar in Wasser ebenfalls heiß zugegossen und unter Umrühren nochmals aufgekocht. Beim Abkühlen soll die Mischung erstarren. Sie wird am besten heiß in die Kulturflaschen eingegossen, so daß der Boden 2 bis 4 *cm* hoch bedeckt wird. Alle diese Nährböden geraten zwar mit der Zeit von selber in Gärung; vorteilhafter ist es jedoch, sie gleich nach Abkühlung zu impfen. Das geschieht durch Aufspritzen einiger Tropfen einer Aufschwemmung von Preßhefe (Bäckerhefe) in lauwarmem Wasser. Nach 24 Stunden (bei Zimmertemperatur nach 12 Stunden bei zirka 28°!) sind die Flaschen bereit für den Gebrauch. Ein gefalteter Streifen Filtrierpapier (5 × 5 *cm* in kleineren Flaschen) wird in jede Flasche gesteckt, um überflüssige Feuchtigkeit aufzusaugen und den Tieren als Sitzgelegenheit zu dienen.

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[1]) Diese scheinen sich im *Morgan*schen Laboratorium gut bewährt zu haben. Wir fügen deshalb das Rezept bei:

Wasser . . . . 71 *g*
Agar . . . . . 1 „
Gelbes Maismehl 16 „
Molasse . . . 7 „
Karo-Maissirup 5 „ oder 12 *g* konzentrierte Zuckerlösung.

Herstellung: 1. Agar gelöst im siedenden Wasser.
2. Zufügen der Molassen.
3. Sorgfältiges Einrühren des Maismehles bis zur Bildung eines homogenen Gemisches.
4. Einfüllen in Flaschen und Impfen mit Hefe.

Pearl (1926) erhielt ausgezeichnete Resultate mit seinem synthetischen Nährmedium, S. 101. Zusammensetzung und Herstellung sind die folgenden:

Lösung A. Rohrzucker . . . . . 500·0 *g*
$KNaC_4H_4O_6\,4H_2O$ . . . . . 50·0 „
$(NH_4)_2SO_4$ . . . . . 12·0 „
$MgSO_4\,7H_2O$ . . . . . 3·0 „
$CaCl_2$ . . . . . 1·5 „

Wasser wird zugesetzt, so daß 3 *l* Lösung erhalten werden.

Lösung B. Agar-Agar . . . . . 135·0 *g*
Weinsäure . . . . . 30·0 „
KH_2PO_4 . . . . . 6·0 „

Wasser wie in Lösung A.

Gleiche Teile der Lösungen A und B werden gemischt und in die Fliegenflaschen eingefüllt.

Das Gedeihen der Drosophila ist, wie das aller Wechselwarmen stark von der Temperatur abhängig. Für Larven und Imagines können 10 und 32° C als die extremen Temperaturgrenzen gelten. Für Zuchtversuche sind 24 bis 25° als Optimum zu betrachten. Das Überschreiten des Spielraumes 17 bis 27° nach unten oder nach oben sollte vermieden werden, weil sonst mit allerhand Unregelmäßigkeiten zu rechnen ist (herabgesetzte Fruchtbarkeit, kurze Lebensdauer, veränderte Austauschraten, veränderte phänotypische Ausprägung mancher Mutanten usw.). Für Forschungszwecke ist darum die Zucht in Thermostaten zu empfehlen; Kursmaterial dagegen kann sehr gut in warmen Zimmern gehalten werden.

Die Lebensdauer der Drosophila variiert beträchtlich. Bei 25° beträgt die larvale Periode ungefähr fünf Tage, die Puppenruhe vier Tage, und für die Imagines mögen 30 Tage als Mittelwert gelten. Einzelne Fliegen sind allerdings auch 80 und 90 (*Lutz*, 1911) oder sogar 153 (*Moenkhaus*, 1911) Tage alt geworden. Andrerseits sterben manche auch viel früher. Nach *Lutz* ist die mittlere Lebensdauer der Weibchen eine geringere als die der Männchen. *Pearl* fand mehrere daraufhin geprüfte Mutanten kurzlebiger als die Wildform.

In der Regel kopulieren die Weibchen in den ersten 24 Stunden nach dem Schlüpfen nicht. Die ersten Eier werden am Ende des zweiten oder zu Beginn des dritten Tages gelegt. Die Zahl der Eier eines Weibchens variiert beträchtlich. In der Literatur sind darüber wenig zuverlässige Angaben zu finden. Die ungeheure Produktivität dieser kleinen Fliegen erhellt aber aus den Tabellen von *Just* (1924), die mehrmals über 1000, sogar über 1200 Nachkommen eines Weibchens verzeichnen. Das Legen erfolgt während der ganzen Periode des adulten Lebens. Praktisch kann mit drei Wochen (bis 25 Tagen) gerechnet werden. Da die Eier sich in zehn Tagen zu Imagines entwickeln, muß bei genetischen Experimenten dafür Sorge getragen werden, daß sich die Generationen in den Kulturgefäßen nicht überschneiden und vermischen. Das kann z. B. nach folgenden Regeln geschehen: 1. Das die Eier liefernde Paar wird nach je zwei bis drei Tagen in eine neue Flasche versetzt. Es ist dann damit zu rechnen, daß je bis zum 17. Tage in jeder Flasche die Mehrzahl der Nachkommen geschlüpft sind, während die zweite Generation noch nicht so weit ist. 2. Das Paar kann je sechs bis sieben Tage in einer Flasche verbleiben, wenn Sorge dafür getragen wird, daß die zweite Generation darin sich nicht fortpflanzt, d. h. vom neunten Tage an müssen aus der Flasche täglich, besser zweimal täglich sorgfältig alle geschlüpften Fliegen entfernt werden. 3. Noch sicherer ist es, wenn bereits die Puppen

entfernt und in neue Gläser übertragen werden. Das kann mittels eines Spatels geschehen, sobald die Puppen durch ihre Bräunung die nötige Reife erkennen lassen. Die Puppengläser brauchen natürlich nicht mit Futter versehen zu sein, dagegen müssen feuchte Papierstreifen eingelegt werden, um das Austrocknen zu verhindern. In der Regel wird man das am wenigsten Zeit beanspruchende Verfahren 2 wählen.

Zum Zwecke der Untersuchung werden die Fliegen mit Äther narkotisiert. Zuerst werden sie aus der Futterflasche in eine reine, trockene Flasche verbracht. Das Umsetzen erfolgt stets mit Ausnutzung der positiven phototaktischen Reaktion: Die beiden Flaschen werden mit den Öffnungen gegeneinander gehalten. Erst ist die Futterflasche zum Licht gekehrt, dann wird gewendet. Durch Klopfen werden sitzende Tiere aufgescheucht. Die Trockenflasche wird nun durch einen Korkstopfen verschlossen, an dessen Innenseite ein kleiner Wattebausch oder ein Stück Filz mit einer Nadel festgeheftet ist. Nachdem hier einige Tropfen Äther aufgeträufelt wurden, wird die Flasche verschlossen. Eine Narkotisierung von einer Minute ist genügend (als Regel kann gelten: 30 Sekunden nach Aufhören der Bewegungen), um die Fliegen ungefähr fünf Minuten ruhig zu haben. Sie werden dann auf ein festes weißes Papier oder auf eine weiße Glasscheibe geschüttet und können nun mit einem weichen Pinsel nach Bedarf herumgewendet werden. Die Untersuchung erfolgt in der Regel unter der Binokularlupe bei ungefähr 15facher linearer Vergrößerung. Wenn zur besseren Beleuchtung eine Lampe verwendet wird, so muß ein Wasserfilter vorgeschaltet werden, um Hitzeschädigungen zu vermeiden. Praktisch erweisen sich die von den optischen Firmen auf den Markt gebrachten festen Glühbirnen-Wasserkugel-Kombinationen.

Die folgenden vier Merkmale gestatten die beiden Geschlechter zu unterscheiden (vgl. auch Fig. 57):

1. Die Weibchen sind merklich größer als die Männchen. Doch variiert die Größe nach Kulturbedingungen.

2. Das Abdomen der Weibchen ist voller als das der Männchen. Ferner ist es gegen das Hinterende schwach birnförmig auslaufend, während es bei Männchen vollkommen abgerundet ist.

3. Die schwarzen Ringe der Hinterleibssegmente sind bei Männchen stärker ausgesprochen, gegen das Ende zu zusammenfließend. Ferner gehen sie rund um den Körper, während die Farbringe der Weibchen ventral offen bleiben.

4. Das Männchen hat Borstenkämme an den Basalgliedern der Vordertarsen. Sie bestehen aus 7 bis 14 „Zähnen" und stellen namentlich bei Mutanten mit heller Körperfarbe das einfachste Kennzeichen des Geschlechtes dar.

Es muß natürlich in allererster Linie darauf geachtet werden, daß zu den Paarungen unbefruchtete Weibchen verwendet werden. Frisch geschlüpfte Tiere, Männchen und Weibchen, sind an der hellen Körperfarbe zu erkennen und insbesondere die Weibchen an dem verlängerten Abdomen. Doch verbürgen solche Kennzeichen durchaus nicht Jungfräulichkeit, namentlich nicht, wenn alte Männchen im Zuchtgefäß waren. Mit gutem Vertrauen dürfen dagegen die Weibchen verwendet werden, wenn die Flaschen halbtägig geleert wurden, d. h. wenn keine Tiere sich darin befinden, die älter als zwölf Stunden sind. Schließlich geht man

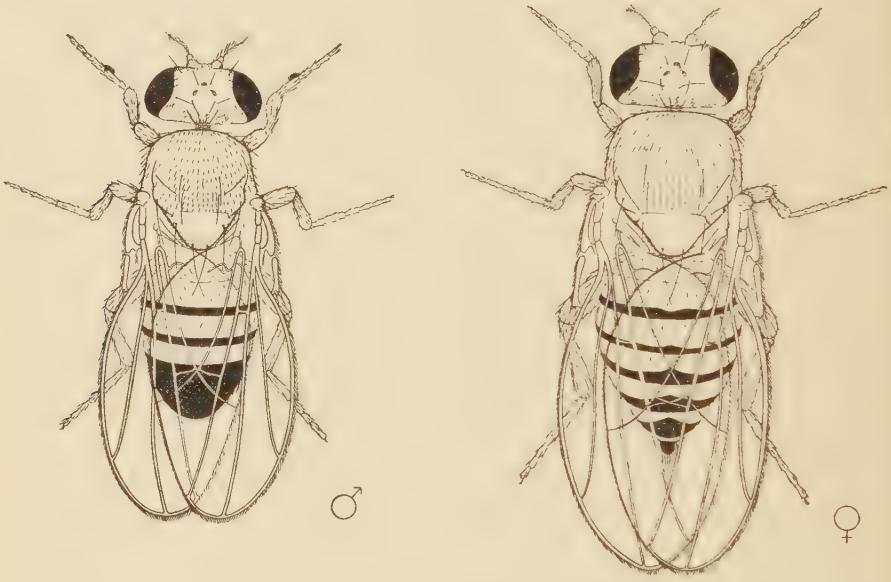


Fig. 57. Männchen und Weibchen von Drosophila melanogaster. (Nach *Morgan*.)

am sichersten, wenn zur Zucht Weibchen verwendet werden, die aus isolierten Puppen geschlüpft sind. Zu dem Zweck werden möglichst große (voraussichtlich weibliche!) Puppen einzeln in 5-cm^3-Röhrchen versetzt und unter Beigabe eines feuchten Papierstreifens mit einem Wattebausch eingeschlossen. Futterbeigabe ist unnütz, da die frischgeschlüpften Fliegen solche noch nicht benötigen. Die Männchen brauchen nicht mit solcher Sorgfalt herausgesucht zu werden, doch wird man darauf achten, daß sie nicht zu alt sind.

Das Einsetzen der narkotisierten Paare in die Kulturgläser erfordert noch einige Sorgfalt. Da die erwachenden Tiere leicht am Futter festkleben können, biegt man sich aus Papier ein

kleines Näpfchen zurecht, legt die Fliegen darauf und setzt es dann oben auf die Futtermasse. Es empfiehlt sich, bei Bastardierungen stets Einzelpaare zu züchten. Auch wenn man von reinen Rassen ausgeht, ist man nie sicher, vollkommen gleichartiges Material zu verwenden. Von Zeit zu Zeit treten ja z. B. Mutationen auf, Fehler können unterlaufen und in all diesen Fällen ist es leichter, Abweichungen vom erwarteten Resultat aus Einzelzuchten heraus zu verstehen. Die Zahl der parallelen und reziproken Zuchten richtet sich natürlich nach der Besonderheit der Aufgabe, doch sollten es mit Rücksicht auf die nicht so seltene Sterilität von Männchen oder Weibchen nie weniger als vier sein.

Die Auszählung der Versuchsresultate erfolgt entweder am frischen oder im Alkohol konservierten Material. Die große Mehrzahl der wichtigeren Mutanten verlieren durch selbst längeres Aufbewahren im Alkohol nicht ihre Kenntlichkeit.

B. Nomenklatur und Schreibweise.

Dem geringen Alter und unentwickelten Zustand der Vererbungswissenschaft entspricht der Mangel einer in allen Teilen konsequent durchgebildeten Nomenklatur. Wertvolle Arbeit auf diesem Gebiete ist wiederum von *Morgans* Laboratorium geleistet worden. Aber nicht alle Grundsätze, die dort eingehalten werden, können auf beliebige Objekte angewendet werden. Drosophila melanogaster ist z. B. nur in einer einzigen Wildform bekannt, und so gilt diese stets als Prototyp, und alle Abweichungen werden als Mutanten bezeichnet. Das kommt in der Schreibweise zum Ausdruck, indem wohl für das mutierte Gen ein Buchstabenzeichen gesetzt wird, für den entsprechenden Wildcharakter dagegen lediglich ein Kreuz oder überhaupt kein Zeichen. Häufig finden wir jedoch in der Natur mehrere derselben Art zugehörige Erbvariationen — oft sogar ohne ausgesprochenes zahlenmäßiges Überwiegen der einen oder anderen. Bei manchen Haustieren wiederum sind Wildformen überhaupt nicht bekannt. Manche Züge der gegenwärtigen Nomenklatur tragen auch notwendig provisorischen Charakter, weil wir über die Physiologie der Vererbung noch so wenig wissen.

Bei Bastardierungen bezeichnet man (nach Vorschlag von *Punnett*) die Generation der (in der Regel reinrassigen) gekreuzten Ausgangstiere als elterliche oder Parentalgeneration (P). Die Nachkommen bilden dann die Tochter- oder Filialgenerationen, welche, fortlaufend numeriert, meist kurz als F_1, F_2, F_3 .... F_n benannt werden. Die Kopulation wird durch ein liegendes Kreuz ($\times$) angedeutet, so daß z. B. die häufig ausgeführte Rückkreuzung der ersten Tochtergeneration

mit einer der Stammformen $F_1 \times P$ geschrieben (lies: „F_1 mal P") wird. Um keinen Zweifel an der Rückkreuzungsnatur bestehen zu lassen, kann R. K. davor gesetzt werden (englisch B. C.). Das Geschlecht wird geschrieben als ♀ (Weibchen), ♂ (Männchen) und ⚥ (Zwitter; weniger gebräuchlich ist ☿). Die vollständige allgemeine Formel für eine Rückkreuzung hat demnach die Form:

$$R.\,K.: F_1 s \times Ps,$$

wobei für s das entsprechende Geschlechtszeichen zu setzen ist.

Die Gene werden mit kurzen, möglichst charakteristischen Namen belegt. Häufig, vielleicht sogar immer sind sie zwar polyphän, d. h. beeinflussen nicht nur einen, sondern mehrere Charaktere. So der „Delta" genannte Faktor von Drosophila, der außer durch die dreieckige Verbreitung der Vereinigungsstellen von Längs- und Randadern der Flügel auch durch kleine rauhe Augen, Borstenmodifikationen usw. ausgezeichnet ist. Recht häufig sind Nebenwirkungen auf physiologische Verhältnisse, wie Lebensdauer, Fruchtbarkeit usw. Man wird dann die Bezeichnung nach dem Merkmal wählen, das die sicherste Identifikation ermöglicht. Wo Doppelnamen nicht zu vermeiden sind, sollen sie stets mit Bindestrich gebunden werden. Das wird in englischen Arbeiten häufiger notwendig sein als in deutschen. (Vgl. white-head = Weißkopf; aber Truncate-intensifier = Gestutzt-verstärker.) Die amerikanischen Autoren schreiben die Namen mit großen oder kleinen Initialen, entsprechend dem vorwiegend dominanten bzw. rezessiven Verhalten. Im Deutschen besteht diesbezüglich keine feste Sitte; es dürfte sich empfehlen, sich dieser Schreibweise ebenfalls zu bedienen. Für die Formeln jedoch ist sie allgemein gebräuchlich. Als Symbole wurden früher oft die Buchstaben in alphabetischer Reihenfolge benutzt. Aus mnemotechnischen Gründen hat man dann die Initialen der Namen verwendet. Noch heute werden solche Zeichen häufig ad hoc gebildet, und *R* mag in einer Arbeit für Rot stehen, in einer anderen für Rund. Der Fortschritt der Forschung macht eine feste Namengebung zur Notwendigkeit. Die Drosophilaforscher (denen hierin mehr und mehr Folge geleistet wird) belegen jede neue Mutation mit Namen und Symbol und fügen bei der Registrierung den Namen des Entdeckers hinzu. Das geschlechtsgebundene Gen für weiße Augenfarbe ist also eindeutig benannt mit white, *w M* (organ). Ein später entdeckter Faktor für weiße Farbe der Ocellen erhielt dann die Bezeichnung white-ocelli, *wo B* (ridges). Mit dem Anwachsen der Zahl der bekannten Gene werden immer häufiger Doppelbuchstaben als Symbole verwendet (*vg* für vestigial usw.). (*Baur* verwendet neuerdings [1924] je drei Lettern, z. B. *Cor* für

die Mutation Cornuta.) Über die Drosophilamutationen werden Register geführt, so daß bei mehrmaligem Auftreten nach den Prioritätsregeln verfahren werden kann.

Namen und Symbole werden bald nach dem dominanten, bald nach dem rezessiven Merkmal gegeben. So ist z. B. white, *w* (*Morgan*) als Augenfarbe bei Drosophila rezessiv; Color *C* (*Castle*), als Grundfaktor für die Farben des Meerschweinchens dagegen dominant. Wie gerade aus diesen Beispielen ersichtlich, belegen manche Forscher die „Wildform" mit Namen, andere die mutative Aberration. Letztere Regel setzt sich neuerdings mehr und mehr durch. Gelegentlich (häufig in botanischen Arbeiten!) werden lateinische Namen gewählt. In konsequenter Weise haben dagegen die Drosophilaforscher auf die lateinische Nomenklatur zugunsten der Muttersprache verzichtet. Es ist noch nicht abzusehen, welches hier die weitere Entwicklung sein wird.

Reine Dominanz bzw. Rezessivität eines Faktors kann eher als Ausnahme gelten. Bastarde zwischen gefärbten und albinotischen Nagern sind fast immer etwas heller als der farbige Elter. Trotzdem betrachtet man Gefärbt (*C*) als dominant, weil in der Regel die Unterschiede nicht auffällig sind, so daß F_2 leichter nach 3:1 als 1:2:1 klassifiziert werden kann. Namentlich ist es aber bei der Analyse von Polyhybriden oportun, soweit möglich nach dem Dominanzschema zu verfahren, weil das Material sonst in zu viele kleine Klassen aufgeteilt werden muß.

Ein Gen kann bekanntlich mehrmals und in verschiedener Weise abändern. Es bildet mit diesen Mutationen zusammen eine Serie multipler Allelomorphs oder unilokaler Faktoren (nach *Baur*; noch weniger gebräuchlich). In der Regel sind sie so beschaffen, daß sie als quantitative Stufen desselben Charakters betrachtet werden können. An einem Ende der Reihe steht eine Mutation, die sich allen anderen gegenüber vorwiegend dominant erweist, am anderen Ende die entsprechend rezessive. Häufig herrscht aber nicht Dominanz, sondern intermediäres Verhalten zwischen den Gliedern solcher Reihen. Allelomorphe Faktoren erhalten stets dasselbe Symbol, dem jedoch dann ein Suffix zur Charakterisierung beigegeben wird. So in *Castles* Color, Serie C (höchster Grad der Färbung), C_d (dilute, verdünnt), C_r (red, rote Augen), C_a (albinotisch in bestimmten Rassen, meist jedoch mit geringen Farbresten), c (vollkommener Albinismus; ist jedoch beim Meerschweinchen noch nicht gefunden worden); oder in *Morgans* white Serie: + (Wildfarbe, rot), w^c (cherry, blaßrot), w^{ec} (eosin), w^{ecc} (écru, gelblich), w (white, weiß). *Wright* bezeichnet übrigens *Castles* „Color" nun als „albino" Serie und schreibt sie mit Einschaltung seines neuen Faktors „dark" in der *Morgan*schen Art: C, c^k, c^d, c^r, c^a. Es besteht sichtlich die Tendenz, sich der

*Morgan*schen Terminologie anzuschließen — ein Bestreben, das durch die überragende Bedeutung der Drosophilaarbeit gerechtfertigt, in Zukunft sich immer mehr geltend machen wird. Konsequenterweise könnten jetzt *C* und *c* noch durch *A* bzw. *a* ersetzt werden, d. h. durch das von *Baur* für kompletten Albinismus der Nager gebrauchte Zeichen.

Manchmal werden auch Zahlen beigegeben. Namentlich ist das gebräuchlich bei quantitativen Analysen, wie sie *Goldschmidt* und *Witschi* an Geschlechtsfaktoren ausführten, wobei Reihen, wie 6 *f*, 9 *f*, 12 *f* usw., aufgestellt wurden.

Morgan ist durch seine reiche Erfahrung zu der Überzeugung gekommen, daß jedes körperliche Merkmal durch eine ganze Serie von Faktoren bestimmt wird. Bei solchem Zusammenwirken von Genen ist dann allerdings die Bedeutung der Komponenten eine wechselnde. Als Polymerie bezeichnete *Lang* das gleichsinnige Wirken von Erbfaktoren, die keine Allelomorphs (Paarlinge nach *Correns*) sind[1]). Solche Fälle sind zuerst von *Nilsson-Ehle* und *East* gefunden worden. Bei Tieren ist namentlich das Körperwachstum schon frühe (durch *Lang* und *Castle*) in diesem Sinne interpretiert worden. *Punnett* findet diese Bedingung auch in der Vererbung der Eifarbe beim Haushuhn. Die klarsten Polymeriefälle kennen wir nun wiederum von Drosophila. Das Rot der Augen wird hier durch eine ganze Reihe von Genen bedingt, welche an 15 verschiedenen Orten der drei großen Chromosomen lokalisiert sind. Das Zusammenwirken dieser Gene erfolgt im allgemeinen im Sinne einer Summation. Je größer die Zahl der Dominanten, desto kräftiger die Farbe und umgekehrt. Nicht selten bringt aber auch schon die eine Dominante allein (sogar im heterozygoten Zustand) den Gesamteffekt hervor. Wenn zwei oder mehr solcher Gene in der Wirkung (auch quantitativ) annähernd identisch sind, so können sie leicht miteinander verwechselt werden und wurden in der Tat zeitweise auch als „mimetische" bezeichnet. Eine Notwendigkeit, sie nomenklatorisch von den polymeren Faktoren zu trennen, besteht jedoch nicht. Fast immer werden polymere Faktoren mit verschiedenen Namen und Symbolen belegt. So im Drosophilafall z. B. white (*w*), vermilion (*v*), safranin (*sf*), scarlet (*st*), pink (*p*) usw. Seltener, etwa bei Studien über Vererbung der Körpergröße, wird dasselbe Symbol, mit Nummern versehen, für alle verwendet. Davon ist entschieden abzuraten, da dieses Verfahren zu Verwechslungen mit Serien multipler Allelomorphs führt. Unter die Rubrik Polymerie fallen wohl auch die typischen Fälle sogenannter „modifizierender Faktoren", die von *Drosophila* beschrieben worden sind. So stellt

[1]) *Punnett, Castle* u. a. sprechen von „multiplen Faktoren". Der Unterschied von „multiplen Allelomorphs" ist wohl zu beachten!

der „*ski*-Flügel" (*Clausen* und *Collins*) ein fast vollkommenes Analogon zum weißspelzigen Hafer (gegenüber schwarzspelzig, *Nilsson-Ehle*) dar. Beide Merkmale werden nur beim Zusammenwirken je zweier Mutationen in verschiedenen Chromosomen manifest. Eine Mutation für sich allein ist phänotypisch nicht erkennbar. Die Drosophilaforscher geben in solchen Fällen beiden Genen denselben Namen und unterscheiden sie lediglich durch Beifügen der Chromosomennummer, z. B. *Ski II* (*Si II*) und *ski III* (*si III*).

Was endlich die Lethalfaktoren der Drosophila betrifft, so werden sie nach drei verschiedenen Methoden benannt: 1. Die im Geschlechtschromosom lokalisierten werden mit arabischen Nummern versehen (*l*-1, *l*-2, *l*-3 . . .). 2. Die, welche im zweiten oder dritten Chromosom liegen, heißen *l II a*, *l II b*, *l II c* . . . bzw. *l III a*, *l III b*, *l III c* . . .). 3. Sofern sie aber im heterozygoten Zustand irgendwie morphologisch kenntlich werden, erhalten sie einen entsprechenden Namen, wie etwa Minute-*a*, Minute-*b* usf.

Wenn ungleichartige Faktoren zusammenwirken, wie in der Fellfarbe von Nagern der Grundfaktor Color (eigentliche Farbbasis, auch etwa „Chromogen" genannt), der Verdunklungsfaktor Black (dessen Fehlen ein starkes Aufhellen der Farbe verursacht, der aber für sich allein farblos bleibt: „Fermentfaktor") und der Verteilungsfaktor Agouti (der ungleiche Farbzonen der Haare bestimmt), so wird das Zusammenwirken in Namengebung und Symbolen nicht angedeutet.

Parallele Gene in verschiedenen Arten oder Genera werden mit gleichem Namen und Symbol belegt. Es ist natürlich in der Regel nicht möglich, zwischen wirklich homologen und lediglich „mimetischen" Faktoren zu unterscheiden, wenn die betreffenden Arten nicht gekreuzt werden können. Trotzdem scheint es berechtigt, gewisse Faktoren etwa der Nagerfellfarbe oder besonders gut (oft auch in der chromosomalen Lokalisation) übereinstimmende Mutationen der verschiedenen Drosophilaspezies identisch zu benennen.

Die summarische Charakterisierung von Tieren erfolgt entweder phänotypisch (nach den homozygoten und den dominanten Merkmalen) oder genotypisch (unter Mitberücksichtigung heterozygoter rezessiver Faktoren, z. B.

genotypisch		phänotypisch	
entweder *AABbcc*	(1)	entweder *ABc*	(4)
oder $\frac{ABc}{Abc}$	(2)		
oder $\frac{+\ c}{bc}$	(3)	oder einfach *c*	(5)

Die Schreibweise (2) wird verwendet, wenn die Herkunft der Gene bekannt ist und festgehalten werden soll. Über den Strich werden die mütterlichen, darunter die väterlichen Gene geschrieben. Reine Homozygoten von der Wildform schreibt *Morgan* einfach mit einem Kreuz (+). Die Vereinfachung der Formeln durch Weglassen der Dominanten ist natürlich nur so weit angängig, als es sich um bekannte Grundfaktoren, ähnlich den Wildfaktoren der Drosophila handelt. Im selben Chromosom liegende (gekoppelte) Faktoren werden näher zusammengerückt oder noch besser durch Bogen verbunden (*ABc hk* oder $\overset{\frown}{ABc}\ \overset{\frown}{hk}$).

C. Freie Kombination.

Bastardierungsexperimente haben in erster Linie die Frage zu entscheiden, ob die untersuchten Charaktere überhaupt nach *Mendel*schen Gesetzen vererbt werden, und wenn ja, wie viele Faktoren dabei beteiligt sind. Ersteres wird im folgenden vorausgesetzt (über nichtmendelistische Vererbung vgl. unten S. 563), und wir wenden uns der Faktorenanalyse zu, zunächst unter Annahme freier Kombination.

Wenn wir die dominierenden Faktoren numerieren und die rezessiven Allelomorphs mit Punkten andeuten, so sind folgendes die sämtlichen möglichen Gen-Kombinationen in den Keimzellen.

1. Monohybridismus (1 Faktorenpaar).

Kombinationen: 1 , . Zahl der Gametensorten: 2.

2. Dihybridismus (2 Faktorenpaare).

Kombinationen: 12 , 1. , .. Zahl der Gametensorten: 4.
.2

3. Trihybridismus (3 Faktorenpaare).

Kombinationen: 123 , 12. , 1.. , ... Zahl der Gametensorten: 8.
1.3 .2.
.23 ..3

4. Polyhybridismus (*n* Faktorenpaare).

Es ist ersichtlich, daß alle die Kombinationen der *n* Dominanten zu den aufeinanderfolgenden Klassen *n*, *n*-1, *n*-2 . . . bis 0 gebildet werden. Da die Frequenz dieser Klassen die binomiale Verteilung zeigt, so kann die Summation aller Kombinationen nach dem Ausdruck $(1 + 1)^n$ erfolgen.

$\Sigma C_{n,n} + C_{n,n-1} + C_{n,n-2} + \ldots\ldots C_{n,0} = 2^n$ Gametensorten.

Da die Merkmalsspaltung bei der Gametenbildung der Bastarde eintritt, so ist theoretisch die Analyse der F_1-Keimzellen oder einer aus solchen sich entwickelnden haploiden Generation der einfachste Weg, um die gewünschten Aufschlüsse zu erhalten. Leider steht er auf zoologischem Gebiet nur selten offen. So z. B. bei parthenogenetisch aus haploiden Eiern sich entwickelnden Hymenopterenmännchen (vgl. unter Haplontenmethoden). Im Prinzip gleiche, d. h. auf Haploidie beruhende Untersuchungsbedingungen bieten die geschlechtsgekoppelten Faktoren (vgl. unten). In weitaus den häufigsten Vererbungsfällen besteht jedoch nur die Möglichkeit, aus den Kombinationen diploider Nachkommengenerationen auf die beteiligten Gene zurück zu schließen. Die moderne Vererbungslehre beginnt bekanntlich mit der Entdeckung dieser Methode durch *Mendel*. Aus der 3:1-Proportion der Dominanten und Rezessiven in F_2 hat er auf ein bei der Reifung spaltendes Faktorenpaar geschlossen und auf die daraus folgende Digametie.

F_2-Analyse.

Bei der Befruchtung der F_1-Bastarde unter sich verbinden sich je zwei Gameten (Ei und Spermium) nach den Gesetzen des Zufalls, d. h. mathematisch gesprochen bilden sich die Variationen mit Wiederholungen zur Klasse 2. Bezeichnen wir die Zahl der Gametensorten mit g, so entstehen die diploiden Faktorenkombinationen der F_2-Generation nach dem Gesetz

$$V'_{g,2} = g^2$$

oder, wenn wir den oben erhaltenen Ausdruck für die Gametenzahl bei n-Faktoren einsetzen, so beträgt die Zahl der Kombinationsweisen in $F_2 = 2^{2n}$, oft auch geschrieben $= 4^n$.

Eine Übersicht über alle Kombinationsmöglichkeiten können wir uns mit dem Kombinationsquadrat verschaffen. Die Fig. 58 stellt ein solches für den Dihybridismusfall dar. Gewählt wurde das klassische *Bateson-Punnett*sche Hybridisationsexperiment mit den vier Kammformen der Hühner. Die Symbole bedeuten R = Rose, P = Pea.

Es ist leicht herauszulesen, daß die Zahl der Homozygoten gleich ist der Zahl der Gameten. Ferner erkennt man sofort, daß die übrigen zwölf Kombinationen teilweise Wiederholungen darstellen, so daß nur neun verschiedene zygotische Kombinationen existieren. Diese fallen wiederum zusammen in nur vier Phänotypen, da die Kombinationsmöglichkeiten der Dominanten durch die Homozygoten bereits erschöpft werden. Die Doppeltdominanten ($R\,P$), die Einfachdominanten (Rp bzw. rP) und die Rezessiven (rp) stehen im Zahlenverhältnis von 9:3:3:1.

Folgende Tabelle (Tab. 5, s. S. 525) stellt in den Kolonnen 1 bis 7 diese Daten übersichtlich zusammen.

Die Verteilungsverhältnisse $(3+1)^n$ ausgeführt, ergeben für

Monohybriden **3 : 1**
D .

Dihybriden **9 : 3 : 3 : 1**
DD D. .D .

Trihybriden **27 : 9 : 9 : 9 : 3 : 3 : 3 : 1**
DDD DD. D.D .DD D.. .D. ..D ...

Tetrahybriden **81 : 27 : 27 : 27 : 27 : 9 : 9 : 9 : 9 : 9 : 9 : 3 : 3 : 3 : 3 : 3 : 1.**
4 D 4mal je 3 D 6mal je 2 D 4mal je 1 D 0 D

Unmittelbar sichtbar sind nur die Phänotypen und so schließen wir denn aus ihrer

RP RP Walnut	RP Rp Walnut	RP rP Walnut	RP rp Walnut
Rp RP Walnut	Rp Rp Rose	Rp rP Walnut	Rp rp Rose
rP RP Walnut	rP Rp Walnut	rP rP Pea	rP rp Pea
rp RP Walnut	rp Rp Rose	rp rP Pea	rp rp Single

Fig. 58. Kombinationsquadrat. (Nach *Punnett*.)

Zahl und ihrer Frequenz rückwärts auf die Zahl der beteiligten Merkmalspaare. Aus acht Phänotypen, die in der Proportion von 27 : 9 : 9 : 9 : 3 : 3 : 3 : 1 auftreten, erkennen wir auf Trihybridismus usw. (Es ist klar, daß innerhalb dieser Verteilungsreihe jeder dominierende Charakter zu seinem rezessiven Allelomorph im Verhältnis von 3 : 1 steht.) Die weitere Analyse verlangt oft die genetische Prüfung einiger F_2-Individuen, namentlich etwa das Heraussuchen der Homozygoten. Ohne weiteres als solche zu erkennen sind nur die Totalrecessiven, d. h. die Vertreter der kleinsten Klasse. Dagegen sind z. B. bei Trihybriden nur ein Siebenundzwanzigstel der Dreifachdominanten homozygot oder entsprechend ein Neuntel der Doppeldominanten, ein Drittel der Einfachdominanten. Der kürzeste Weg über die genotypische Konstitution solcher Individuen Klarheit zu bekommen, besteht in ihrer Kreuzung mit Totalrecessiven.

TABELLE 5.

1. Zahl der Merkmalspaare	1	2	3	4	5	10	n
2. Zahl der Gametensorte in F_1	2	4	8	16	32	1024	2^n
3. Zahl der Kombinationsweisen in F_2 . .	4	16	64	256	1024	1,048.576	$2^{2n} = 4^n$
4. Zahl der ungleich konstituierten Genotypen in F_2 (zygotisch) . . .	3	9	27	81	243	59.049	3^n
5. Zahl der Homozygoten in F_2	2	4	8	16	32	1024	2^n
6. Zahl der verschiedenen Phänotypen in F_2 bei reiner Dominanz . .	2	4	8	16	32	1024	2^n
7. Frequenz der Phänotypen bei reiner Dominanz	$(3+1)^1$	$(3+1)^2$	$(3+1)^3$	$(3+1)^4$	$(3+1)^5$	$(3+1)^{10}$	$(3+1)^n$
8. Zahl der Phänotypen in F_2 bei intermediärem Verhalten .	3	9	27	81	243	59.049	3^n
9. Frequenz der Phänotypen bei intermediär. Verhalten	$(1+2+1)^1$	$(1+2+1)^2$	$(1+2+1)^3$	$(1+2+1)^4$	$(1+2+1)^5$	$(1+2+1)^{10}$	$(1+2+1)^n$

Homozygote geben dann uniforme Nachkommenschaften, während alle Heterozygoten aufspalten. Dieses Heraussuchen der Homozygoten, das in der züchterischen Praxis natürlicherweise eine große Rolle spielt, wird bedeutend erleichtert, wenn die Dominanz keine ganz reine ist, so daß die Homozygoten mit einiger Sicherheit schon an der Klarheit ihrer Charakterprägung erkennbar werden.

Wir haben bisher den Ausführungen die Annahme des Dominanzverhältnisses zugrunde gelegt. Wenn nun aber Heterozygoten ausgesprochen intermediär sind oder sogar (gleich den blauen Andalusiern) einen neuartigen Typus zeigen, so wird natürlich die Zahl der Phänotypen auf der Basis von 3^n erhöht und ihre Frequenz berechnet sich nach der Formel $(1+2+1)^n$. (Vgl. Kolonnen 8 und 9 der Tab. 5, S. 525). Sind m dominant und p intermediär vererbende Merkmalspaare beteiligt, so gelten entsprechend $2^m \cdot 3^p$ bzw. $(3+1)^m\ (1+2+1)^p$. Wie oben bereits gesagt, sucht man, wenn irgend möglich, die Betrachtung nach dem Dominanzverhältnis durchzuführen.

Wenn alle Merkmale eines Kreuzungsfalles unabhängig voneinander zur Ausprägung gelangen, so ist die Analyse eine verhältnismäßig einfache Sache. Kompliziert gestaltet sie sich dagegen, wenn Faktoren zusammenwirken — sei es im Sinne der Verstärkung oder Abschwächung (Polymerie) oder so, daß die Ausprägung eines Merkmals die Anwesenheit eines anderen zur Voraussetzung hat. Beginnen wir mit dem letzteren Fall, der abweichende Zahlenverhältnisse in F_2 verursacht. Bei gewissen Linien von Nagern bestimmt der Grundfaktor für Fellfarbe *C* (Color) gelbes Pigment. Dazu kann sich ein weiterer Faktor *E* (Extension) gesellen, der die Farbe zu Schokoladebraun eindunkeln läßt. In F_2 erwarten wir nach dem Dihybridismusfall folgende Phänotypen und Frequenzen:

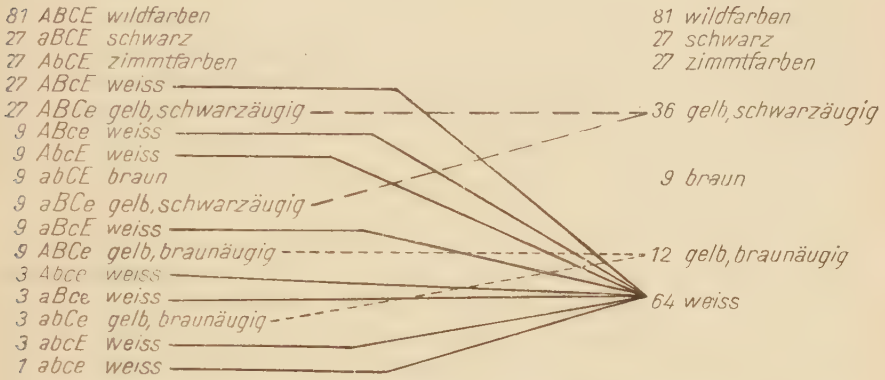
$$9\ CE : 3\ Ce : 3\ cE : 1\ ce.$$

Nun ist aber der Verdunklungsfaktor bei Abwesenheit des Grundfaktors wirkungslos. Die Kombinationen *cE* sind demnach phänotypisch identisch mit *ce*, d. h. albinotisch. So erscheinen also nur drei Phänotypen, und zwar im Zahlenverhältnis von

9 Braun : 3 Gelb : 4 Weiß.

Interessant ist ein von *Castle* untersuchter Tetrahybridismusfall bei einer Kreuzung unseres domestizierten Meerschweinchens mit Cavia cutleri, seiner möglichen peruanischen Stammform. Außer den Faktoren *C* und *E* wirkten dabei noch *B* (*Black*) und *A* (*Agouti*) mit. *BCE*-Tiere sind schwarz, *BC*-Tiere dagegen gelb

mit schwarzen Augen (*C*-Tiere sind gelb mit braunen Augen!). Der Agoutifaktor wird nur sichtbar im Zusammenspiel mit *CE*, und zwar so, daß *ACE*-Kombinationen zimtfarben, *ABCE* dagegen wildfarben werden. *C* ist natürlich immer Grundbedingung für das Erscheinen irgendeiner Farbe überhaupt. Statt 16 resultieren nun aus diesem Tetrahybridismusfall nur sieben Phänotypen in stark vom Schema abweichenden Zahlenproportionen, wie aus nachstehender Zusammenstellung zu ersehen ist.



Es erübrigt sich kaum zu bemerken, daß solche Fälle kummulierten Zusammenwirkens der Analyse größte Schwierigkeiten bereiten, wenn sie neu in Angriff genommen werden. Die Anwesenheit eines Grundfaktors für Färbung ist allerdings ohne weiteres erkenntlich, da die Gesamtzahl der Farbigen zu den Weißen sich wie 3: 1 stellt. Unter den Farbigen allein finden wir noch sechs Phänotypen, eine Zahl zwischen vier und acht, was auf die Beteiligung von weiteren wenigstens drei Faktoren schließen läßt, und auf ein weiteres abhängiges Verhalten von wenigstens zwei derselben. Eine weitere direkte Analyse wird meistens daran scheitern, daß die Individuenzahlen solcher Polyhybriden zu klein sind, um die relativen Frequenzen der Klassen mit Sicherheit erkennen zu können. In der Regel wird man ja nicht die Untersuchungen mit Polyhybriden, sondern wenn immer möglich mit Monohybriden beginnen und so nach und nach die einzelnen Faktoren kennenlernen. Sonst steht dann immer der Weg offen, aus der F_2 die einzelnen Typen herauszugreifen und durch Rückkreuzungen (s. u.) weiter zu analysieren.

Bei gleichsinnigem Zusammenwirken von Faktoren (Polymerie) können folgende Fälle unterschieden werden:

1. Jeder Faktor bringt für sich allein den vollen Effekt hervor. Falls die Faktoren dominant sind, werden alle F_2-Nachkommen,

mit Ausnahme der Totalrecessiven den Charakter zeigen. Sind sie dagegen recessiv, so erscheint er nur in den Phänotypengruppen, welche im einen oder im anderen homozygot (= recessiv) sind, d. i. in allen außer der ersten. Die erste dieser Alternativen ist realisiert in einer Haferkreuzung mit zwei dominanten Schwarzfaktoren. Das Resultat ist 15 Schwarz : 1 Weiß. Die zweite ergibt sich aus der Kreuzung der beiden schwarzen Drosophilamutanten black und ebony, mit dem Resultat in F_2 von 9 wildfarbenen: 7 schwarzen. (Bei genauem Zusehen werden in der Regel die „gleichartigen" Faktoren noch geringe Unterschiede zeigen; so z. B. im eben erwähnten Drosophilabeispiel. Sie können aber so gering sein, daß die saubere Trennung praktisch zur Unmöglichkeit wird.) In beiden Fällen ist das Zahlenverhältnis recht charakteristisch; noch kennzeichnender ist das Auftreten von Neukombinationen — hier weiß bzw. wildfarben — aus der Kreuzung je zweier reinzüchtender schwarzer Stämme.

2. Die Wirkung der Faktoren addiert sich. Am einfachsten gestaltet sich dieser Fall, wenn sich damit das intermediäre Verhalten verbindet und alle Faktoren mit ungefähr gleicher Stärke wirken. Im allgemeinen werden diese Bedingungen heute für das Körperwachstum als geltend angenommen. Die Phänotypen in F_2 zeigen dann eine binomiale Verteilung, wie aus dem folgenden suponierten Beispiel erhellt. Eine große Rasse *AABBCC* werde gekreuzt mit der kleinen *aabbcc*. Die F_1 ist dann mittelgroß, weil *AaBbCc*. Sie bildet $2^3 = 8$ Gametensorten mit drei, zwei, einem oder null dominanten Faktoren, und zwar leicht ersichtlich in der Proportion der Binomialkoeffizienten von $(1 + 1)^3$, also

$$\text{Gameten } 1\ (3\ D) : 3\ (2\ D) : 3\ (1\ D) : 1\ (0\ D).$$

Durch freie Kombination dieser Gameten entstehen F_2-Zygoten mit sechs bis null Dominanten in der relativen Häufigkeit, welche der Binomialreihe $(1 + 1)^6$ entspricht, also Zygoten

$$1\ (6\ D) : 6\ (5\ D) : 15\ (4\ D) : 20\ (3\ D) : 15\ (2\ D) : 6\ (1\ D) : 1\ (0\ D).$$

Am häufigsten sind die mittleren Größen vertreten, während die extremen Varianten verhältnismäßig selten sind. Infolge der gerade in der Größenentwicklung so allgemeinen individuellen Lebenslageschwankungen werden die Unterschiede zwischen den sieben Klassen mehr oder weniger verwischt. Wenn wie im gewählten Beispiel relativ wenige Faktoren beteiligt sind, so können eventuell die Klassen kenntlich bleiben und durch ihre Zahl (k) den Rückschluß auf die Anzahl der beteiligten Faktoren (n) gestatten nach der Formel $n = \frac{k-1}{2}$. Der erste Eindruck eines

so beschaffenen Materials wird stets der einer kontinuierlichen fluktuierenden Variation sein. Zu seiner Analyse müssen möglichst kleine Klassenspielräume gewählt werden und so werden dann, wenn überhaupt, die verschiedenen Maxima zum Vorschein kommen. Bei graphischer Darstellung muß eine mehrgipflige Kurve mit im allgemeinen binomialem Verlauf resultieren. Aus einer derartigen fünfgipfligen Kurve hat *Davenport* geschlossen, daß an der Vererbung des schwarzen Pigments der Mulatten zwei Faktorenpaare beteiligt sind.

Es ist natürlich durchaus nicht immer der Fall, daß, wie oben angenommen, die eine der Ausgangsformen alle dominanten und die andere alle recessiven polymeren Faktoren enthält. *Punnett* fand z. B. bei der Kreuzung einer großen Hühnerrasse mit einer

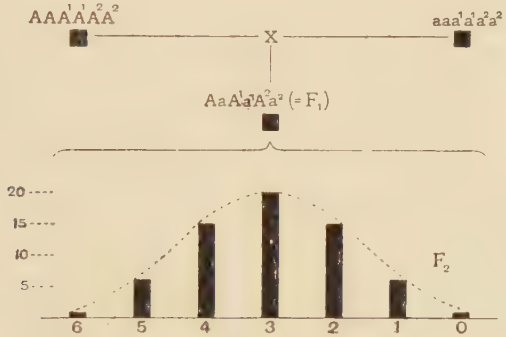


Fig. 59. Graphische Darstellung des Vererbungsganges, wenn drei polymere (multiple) Faktorenpaare beteiligt sind. Die Zahlen auf der linken Seite der F_2-Gruppe beziehen sich auf die Anzahl der Individuen.
(Nach *Punnett*.)

kleinen in F_2 eine Variabilität, die nach oben und unten die Masse der Ausgangsrassen überschritt. Folgerichtig kommt er zu folgender Interpretation des Falles. Die große Ausgangsrasse führt drei dominante Faktorenpaare, die kleine Rasse nur eines. Die erstere ist zu schreiben *AABBCCdd*, die zweite *aabbccDD*. In F_2 werden dann Kombinationen mit sieben und acht bzw. mit einem oder gar keinen Größenfaktoren auftreten und damit die parentalen Variationsgrenzen überschreiten. Die Fig. 59 zeigt die Ausführung eines Polymeriefalles in der gebräuchlichen graphischen Methode.

Kompliziert wird die Untersuchung des Zusammenwirkens ungleich starker Faktoren oder von dominanten mit recessiven. *Punnett* hat solche Fälle (Färbung der Eischalen) zu analysieren versucht, die durch zweigipflige Verteilungskurven in F_2 charakterisiert waren. Eine allgemeine Methodik gibt es hier nicht, sondern von Fall zu Fall wird versucht, durch eine auf freien Ansätzen

konstruierte Kurve dem tatsächlichen Verteilungsbild möglichst nahezukommen.

Den Polymeriefällen anzuschließen sind die modifizierenden Faktoren — auch einfach Modifikatoren genannt. Man hat ihnen neuerdings im Zusammenhang mit dem Selektionsproblem und der Frage der Konstanz der Gene vermehrte Aufmerksamkeit geschenkt. Ihre Wirksamkeit besteht in der Verstärkung oder Abschwächung anderer Faktoren. Im letzteren Falle werden sie auch Abschwächer oder Inhibitoren genannt. Wenn sie nicht mit ihrem spezifischen Faktor zusammen auftreten, so sind sie in der Regel ohne irgendwelche Wirkung. Das klassische Beispiel sind *Castles* gescheckte sogenannte Haubenratten. Durch Selektion konnte eine hochgradige Reduktion der gefärbten Bezirke erreicht werden. *Castle* nahm erst an, der Scheckungsfaktor selber habe sich verändert. Als er jedoch die selektierten Tiere mit ganzfarbenen rückkreuzte, kehrten die von F_2 weg herausmendelnden Schecken wieder zum Typus zurück. *Castle* schließt daraus, daß die Selektion lediglich eine Häufung von Modifikatoren erreichte, die bei der Auskreuzung mit Ganzfarbenen wiederum teilweise abspalteten. An Drosophila konnten einige derartige Modifikatoren bestimmt gefaßt werden, z. B. fand *Lancefield* den Faktor „semi-forked", der für sich allein kaum irgendwelche Wirkung ausübt, dagegen den recessiven Faktor „forked" im heterozygoten Zustand unterstützt, so daß er intermediär zwischen „normal" und „forked" zur Erscheinung kommt. Forked ist im ersten, der Modifikator im dritten Chromosom lokalisiert. Die beiden können darum in der Tat durch Auskreuzen leicht voneinander getrennt werden. Diese Methode des Auskreuzens mit irgendwelchen nicht selektierten Stocks oder überhaupt fremden Linien wird jetzt in der Regel gebraucht, um zu prüfen, ob eine phänotypische Abänderung eines Merkmals auf der Mutation des Grundfaktors oder auf dem Eingreifen von Modifikatoren beruht. Durchaus befriedigen kann sie natürlich nicht, da ja die eingekreuzte fremde Rasse bezüglich ihrer eigenen Modifikatoren unanalysiert ist.

Rückkreuzungsanalyse.

Die Methode der F_2-Analyse hat den Vorteil breitester Anwendungsmöglichkeit und gibt sicherste Auskunft über alle in einem Kreuzungsfalle beteiligten Faktoren. Sie ist aber auch mit zwei Nachteilen behaftet, die oft recht unangenehm werden können. Zunächst haben wir bereits erwähnt, daß die individuellen Konstitutionen der Phänotypenklassen mit dominanten Eigenschaften nicht direkt erkennbar sind. Vor allem aber ist die unsymmetrische

Verteilung der Individuen auf die verschiedenen Klassen für die statistische Bearbeitung sehr unvorteilhaft. Schon bei Trihybriden finden wir die Totalrecessiven nur noch mit einem Vierundsechzigstel in der Gesamtnachkommenschaft vertreten. Die Gefahr, diese Klasse überhaupt nicht zu finden, ist bei beschränktem Material und hoher Faktorenzahl stets vorhanden. Aus diesem Grunde wird in vielen Fällen die Rückkreuzungsmethode bevorzugt.

Ursprünglich versteht man unter Rückkreuzung die Kombination eines F_1-Hybriden mit einem P-Elter. Doch wird der Begriff auch weiter gefaßt, da nicht nur mit Angehörigen der Elterrassen, sondern auch mit rassenfremden Tieren bekannter Konstitution „rückgekreuzt" wird. Wesentlich ist, daß Heterozygoten mit recessiven Homozygoten zur Befruchtung kommen.

In typischen Fällen, d. h. wenn jeder beteiligte Faktor zu selbständiger Ausprägung befähigt ist, erscheinen in der Rückkreuzungszucht alle Kombinationen in gametischer Proportion. Also bei Monohybriden

$$1\ D : 1.$$

oder bei Trihybriden

$$1\ DDD : 1\ DD. : 1\ D.D : 1.DD : 1\ D.. : 1.D. : 1..D : 1...$$

Vorausgesetzt, daß keine phänotypisch sich verdeckenden oder multipel bedingten Faktoren beteiligt sind, kann die genetische Beschaffenheit eines jeden Tieres ohne weiteres aus seinem Habitus abgelesen werden. Es ist klar, daß es in diesen Zuchten außer den rein Recessiven keine Homozygoten gibt. Die Rückkreuzungsmethode findet vor allem bei der Bestimmung der Faktorenaustauschwerte ausgiebigste Anwendung, wie wir noch sehen werden.

Haplontenmethoden.

Botanische Objekte mit ausgedehnter Haplophase haben sich neuerdings für das direkte Studium der Faktorenspaltung als günstig erwiesen. Auch im Tierreich stehen diesbezüglich Möglichkeiten offen und durch die Verbesserung der Methoden zur Auslösung künstlicher Parthenogenese können sie wahrscheinlich noch erweitert werden. Zu brauchbaren Resultaten haben allerdings bis jetzt nur Arbeiten mit Hymenopteren geführt, bei denen sich bekanntlich die Männchen normalerweise aus unbesamten Eiern mit haploider Chromosomenzahl entwickeln. *Perez* (1879), *Cuénot* (1909) und *Newell* (1914) kreuzten gelbe, schwarze und graue Honigbienen. Die Resultate sind nicht immer eindeutig und die Sitte des Hochzeitsfluges erschwert die Kontrolle der wichtigsten Phase des Experimentes. So bleibt vorläufig als einzig gesichert die

interessante Arbeit von *Whiting* (1918—1926) mit der parasitischen Wespe Habrobracon bestehen. (Über die Zuchtmethoden, vgl. Wh. 1921.) Als demonstratives Beispiel sei die Kreuzung von schwarz- und organgeäugigen Mutanten herausgegriffen. Da schwarz dominiert, sind F_1-Weibchen stets schwarzäugig. F_1-Männchen dagegen schlagen nach der Mutter, von der allein sie ihr Erbgut beziehen. Reziproke Kreuzungen fallen daher ungleich aus:

P: Schwarz ♀ × orange ♂
F_1: Weibchen und Männchen schwarz

oder reziprok

P: orange ♀ × schwarz ♂
F_1: Weibchen schwarz, Männchen orange.

Als Haplonten sind die F_1-Männchen natürlich homogametisch. Dagegen sind die F_1-Weibchen beider Kreuzungen Heterozygoten. Unbefruchtet isoliert liefern solche zu gleichen Teilen schwarzäugige und orangeäugige Männchen; z. B. erhielt *Whiting* (1921) von 26 Bastardweibchen 967 schwarz- und 996 orangeäugige Männchen, einer der schönsten Demonstrationen der Mendelspaltung.

Die mathematische Auswertung der Zuchtergebnisse.

Um das Zusammenstimmen der Bastardierungsergebnisse mit möglichen Mendelproportionen zu prüfen, sind drei Formen ihrer Darstellung gebräuchlich.

1. Die prozentuale Berechnung ist nur empfehlenswert, wenn Aufspaltungen in einfachen Verhältnissen wie 1:1 (50% + 50%) oder 3:1 (75% + 25%) vermutet werden. Namentlich gern werden Sexualverhältnisse so ausgedrückt. Beispiel: Von 196 amerikanischen Waldfröschen waren 100 Weibchen und 96 Männchen. Das sind 51% ♀ und 49% ♂.

2. Die Berechnung der relativen Verhältniszahlen hat sich stets auf die Gesamtkombinationszahl zu beziehen, also bei Monohybriden auf 4, bei Dihybriden auf 16 usw. Wenn eine Zucht 282 Dominante und 90 Recessive ergibt, so ist demnach das Verhältnis nicht mit 3 : 0·96 anzugeben (Summe 3·96 statt 4!). Sie muß vielmehr als $\frac{4}{372} \cdot 282 : \frac{4}{372} \cdot 90 =$ = 3·03 : 0·97 berechnet werden, d. h. nach der allgemeinen Form

$$\frac{\text{Kombinationszahl}}{\text{Gesamtindividuenzahl}} \cdot \text{Klassenzahl}.$$

3. Die ideale Verteilungsreihe oder die theoretische Erwartung in absoluten Zahlen

wird in einer entsprechenden Weise festgestellt und mit dem empirischen Ergebnis verglichen. Der Reihe 290 *DD* + 96 *D.* + + 90 *.D* + 28 .. liegt das Spaltungsverhältnis der Dihybriden offensichtlich zugrunde. Die theoretisch zu erwartende Anzahl der Individuen jeder Klasse berechnet sich dann als

$$\frac{\text{Gesamtindividuenzahl}}{\text{Kombinationszahl}} \text{ . relative Klassenzahl.}$$

Im gewählten Beispiel also

$$\frac{504}{16} \cdot 9 + \frac{504}{16} \cdot 3 + \frac{504}{16} \cdot 3 + \frac{504}{16}$$

Zum Vergleich werden dann die beiden Reihen nebeneinander gestellt.

Empirisches Resultat: 290 *DD* + 96 *D.* + 90 *.D* + 28 ..
Theoretische Erwartung: 283,5 *DD* + 94,5 *D.* + 94,5. *D* + 31,5 ..

Häufig wird der einfache Vergleich des emperischen Versuchsergebnisses mit den theoretischen Zahlen dem Forscher genügen, um die Möglichkeit des Zusammenstimmens abzuschätzen. Sicherer ist es stets, sich dabei der einfachen mathematischen Fehlermethoden zu bedienen. Die deutschen Forscher pflegen in diesem Falle den sogenannten „mittleren Fehler" zu bestimmen. Kennzeichnender wäre das Maß allerdings als „Standardfehler" zu bezeichnen, da es auf der Standardabweichung (σ) als Maß der Variabilität basiert. Der mittlere Fehler (m) bestimmt sich nämlich als $m = \pm \frac{\sigma}{\sqrt{n}}$, worin n die Zahl aller Individuen bedeutet (vgl. S. 503). Nun bestimmt man in bequemster Weise den mittleren Fehler von Mendelzahlen für die verschiedenen Klassen gesondert. Im Dihybridismusfall also zuerst für die Doppeltdominanten (Verhältnis 9:7), dann für die Einfachdominanten (3:13) und endlich für die Rezessiven (1:15). Bei alternativer Variation, wie sie hier vorliegt, gilt nun für die Standardabweichung der einfache Ausdruck

$$\sigma = \frac{\sqrt{p_1 \cdot p_2}}{n}$$

in dem p_1 und p_2 für die theoretischen Frequenzen der beiden Klassen stehen. Das Resultat erscheint in Bruchteilen von 1. Wir ziehen aber wiederum vor, unsere Berechnungen auszuführen, entweder 1. in Prozenten, 2. in Teilen der Kombinationszahl oder 3. in absoluten Zahlen. Entsprechend haben wir

den Wert für σ mit 100, mit K oder mit n zu multiplizieren und erhalten dann

$$\text{ad 1. } \sigma_{\%} = \frac{100\sqrt{p_1 \cdot p_2}}{n} = \sqrt{p_1\,\% \cdot p_2\,\%}$$

$$\text{ad 2. } \sigma_K = \frac{K\sqrt{p_1 \cdot p_2}}{n} = \sqrt{K\frac{p_1}{n} \cdot K\frac{p_2}{n}}$$

Wie leicht ersichtlich, bedeuten im zweiten Ausdruck die beiden Faktoren unter der Wurzel nichts anderes als die theoretischen relativen Verhältniszahlen (also 3 bzw. 1 im Monohybridismusfall). Wir setzen dafür v_1 bzw. v_2, so daß wir einfacher schreiben können

$$\sigma_K = \sqrt{v_1 \cdot v_2}$$

$$\text{ad 3. } \sigma_{\text{abs.}} = \frac{n\sqrt{p_1 \cdot p_2}}{n} = \sqrt{p_1 \cdot p_2}$$

Zur Berechnung der mittleren Fehler kann jetzt in der Formel

$$m = \pm\frac{\sigma}{\sqrt{n}}$$

für σ jeweils der entsprechende abgeleitete Wert eingesetzt werden. Da die Ausdrücke aber essentiell in allen dreiFällen gleich lauten, so können wir allgemein schreiben

$$m = \pm\sqrt{\frac{k_1 \cdot k_2}{n}}$$

In dieser endgültigen Formel stehen k_1 und k_2 für die beiden theoretischen Klassenwerte. Der mittlere Fehler erscheint in Einheiten von Prozenten, Verhältniszahlen oder absoluten Individuenzahlen, entsprechend den Benennungen von k_1 und k_2. Ihre Anwendung auf die drei oben berechneten Beispiele mag das illustrieren.

Beispiel 1. Theoretisch sind 50% Weibchen und 50% Männchen zu erwarten. Bei einer Gesamtindividuenzahl von 196 beträgt demnach der mittlere Fehler

$$m = \pm\sqrt{\frac{50 \cdot 50}{196}} = \pm 3{\cdot}6\,\%$$

Da die empirische Abweichung nur 1·0% beträgt, ist die Übereinstimmung des Ergebnisses mit der Norm eine gute.

Beispiel 2. Das theoretische Verhältnis ist 3:1. Bei 372 Individuen ist der mittlere Fehler demnach

$$m = \pm\sqrt{\frac{3 \cdot 1}{372}} = \pm 0{\cdot}09$$

Das zu erwartende relative Verhältnis der Dominanten und Rezessiven ist somit $3 \pm 0{\cdot}09 : 1 \mp 0{\cdot}09$. Die Abweichungen der empirischen Zahlen liegen auch hier innerhalb der einfachen Fehlergrenzen.

Beispiel 3. Hier ist jede Gruppe mit der Summe aller anderen zu vergleichen. Die mittleren Fehler betragen darum der Reihe nach

$$\pm\sqrt{\frac{283{\cdot}5 \cdot 220{\cdot}5}{504}} = \pm 11{\cdot}1$$

$$\pm\sqrt{\frac{94{\cdot}5 \cdot 409{\cdot}5}{504}} = \pm 8{\cdot}8$$

desgleichen $= \pm 8{\cdot}8$

$$\pm\sqrt{\frac{31{\cdot}5 \cdot 472{\cdot}5}{504}} = \pm 5{\cdot}4$$

Die mittleren Fehler sind hier in absoluten Zahlen gegeben (Individuen!). Sie werden den Zahlen der theoretischen Erwartung direkt beigefügt ($283{\cdot}5 \pm 11{\cdot}1\ DD$ usw.) oder in besonderer Zeile unter die ideale Zahlenreihe geschrieben.

Statt des mittleren Fehlers wird auch der „wahrscheinliche Fehler" berechnet. Die englischen und amerikanischen Genetiker, welche dieses Maß bevorzugen, haben dafür den Namen „Probable Error" und das Symbol *P. E.* Wie der mittlere Fehler auf der Standardabweichung, so basiert der *P. E.* auf dem Quartilswert. Die beiden können darum jeder in den anderen transformiert werden nach den Bezugsgleichungen

$$P.\ E. = 0{\cdot}6745\ m$$

bzw.

$$m = 1{\cdot}483\ P.\ E.$$

Die folgende Zusammenstellung nach dem oben gewählten dritten Beispiel mag diese Beziehungen nochmals illustrieren.

Theoretische Erwartung:	$283{\cdot}5\ DD$	$+ 94{\cdot}5\ D.$	$+ 94{\cdot}5\ .\ D$	$+ 31{\cdot}5$ ..
m:	$\pm 11{\cdot}1$	$\pm 8{\cdot}8$	$\pm 8{\cdot}8$	$\pm 5{\cdot}4$
P. E.:	$\pm 7{\cdot}5$	$\pm 5{\cdot}9$	$\pm 5{\cdot}9$	$\pm 3{\cdot}6$
Empirisches Resultat:	$290\ DD$	$+ 96\ D.$	$+ 90\ .\ D$	$+ 28$ ..

Was besagen nun diese Fehlerbestimmungen und welche Sicherheiten geben sie betreffend die Richtigkeit einer Interpretation?

Bezeichnen wir die theoretische Erwartung mit *T. E.* So umfaßt das Intervall *T. E.* $\pm$ *P. E.* gerade 50% und das Intervall *T. E.* $\pm$ 4 *P. E.* 99·3% der empirischen Fälle, immer vorausgesetzt, daß keine anderen Faktoren als die der reinen Zufallsvariation die Resultate beeinflußt haben. Wir sehen also, daß es in einem einzelnen Falle genau eben so wahrscheinlich ist, daß der Wert des wahrscheinlichen Fehlers über- als unterschritten werde. Praktisch muß jedoch das Resultat innerhalb der Grenzen des vierfachen wahrscheinlichen Fehlers bleiben.

Der mittlere Fehler ist ein weiteres Maß, so daß in den Raum *T. E.* $\pm$ *m* bereits 68·3% und in das Intervall *T. E.* $\pm$ 3 *m* schon 99·7% — d. h. praktisch alle Fälle — eingeschlossen sind.

Die Fehlerbestimmungen sind eine wertvolle Hilfe, da sie allein ein objektives Vergleichen verschiedener Versuchsresultate ermöglichen. Dagegen kann man von ihnen allein nicht die Entscheidung darüber verlangen, ob eine versuchte Interpretation richtig sei. Der Natur der Sache entsprechend geben sie nur über die mathematische Zulässigkeit einer solchen Aufschluß. Liegen Werte außerhalb $\pm$ 3 *m* oder $\pm$ 4 *P. E.*, so ist anzunehmen, daß entweder die mendelistische Interpretation unrichtig sei oder aber sekundäre biologische Faktoren — namentlich solche, welche die Vitalität beeinflussen — abändernd einwirkten. Bei Versuchen mit kleiner Gesamtindividuenzahl ist auch in Betracht zu ziehen, daß Verhältnisse wie 1:1, 9:7, 3:1 sich zum Teil schon mit den einfachen und erst recht mit den drei- bzw. vierfachen Fehlergrenzen überschneiden. In nicht unmittelbar klaren Fällen wird man deshalb Parallelversuche anstellen oder Testproben machen, von denen die Kombination von F_2- und *R.-K.*-Zuchten, schon von *Mendel* verwendet, noch heute die meist geübte ist.

Wer häufig Fehlerbestimmungen auszuführen hat, wird sich gern der Tabellen bedienen, die von verschiedenen Autoren ausgearbeitet worden sind. Wir lassen hier eine solche betreffend relative Verhältniszahlen folgen (Tab. 6). Ausführliche Tabellen finden sich bei *Castle* (1924), *Emerson* (1922), *Pearson* (1900).

D. Faktorenkoppelung, Austausch und Rekombination.

Als zu Beginn dieses Jahrhunderts Vererbungsexperimente auf größerer Basis durchgeführt wurden, wurde bald offensichtlich, daß das *Mendel*sche Gesetz der freien Kombination der Gene nicht durchhaltend anwendbar ist. In steigender Zahl kamen Fälle zur Beobachtung, in denen gewisse Kombinationen konstant häufiger und andere Kombinationen entsprechend seltener gebildet werden, als nach den Zufallsregeln erwartet werden müßte.

TABELLE 6.

Tabelle der mittleren Fehler der relativen Verhältniszahlen für verschiedene Individuenzahlen (zum Teil nach *W. Johannsen*).

Zahl der Kombinationen	Theoretisches Verhältnis	Mittlerer Fehler bei der Individuenzahl						
		n = 25	n = 50	n = 100	n = 250	n = 500	n = 1000	n = 2000
2	1 : 1	0·2000	0·1414	0·1000	0·0633	0·0447	0·0316	0·0224
4	3 : 1	0·3464	0·2449	0·1732	0·1095	0·0775	0·0548	0·0388
	2 : 2	0·4000	0·2828	0·2000	0·1265	0·0894	0·0632	0·0447
16	15 : 1	0·7746	0·5477	0·3873	0·2450	0·1732	0·1225	0·0866
	13 : 3	1·2490	0·8832	0·6245	0·3950	0·2793	0·1975	0·1397
	12 : 4	1·3856	0·9798	0·6928	0·4382	0·3098	0·2191	0·1549
	9 : 7	1·5875	1·1225	0·7937	0·5020	0·3550	0·2510	0·1775
64	63 : 1	1·5875	1·1225	0·7937	0·5020	0·3550	0·2510	0·1775
	62 : 2	2·2271	1·5748	1·1136	0·7043	0·4980	0·3522	0·2490
	61 : 3	2·7055	1·9131	1·3528	0·8556	0·6050	0·4278	0·3028
	60 : 4	3·0984	2·1909	1·5492	0·9798	0·6928	0·4899	0·3464
	58 : 6	3·7310	2·6382	1·8655	1·1799	0·8343	0·5899	0·4172
	55 : 9	4·4497	3·1465	2·2249	1·4072	0·9950	0·7036	0·4975
	48 : 16	5·5426	3·9192	2·7713	1·7527	1·2394	0·8764	0·6197
	46 : 18	5·7550	4·0694	2·8775	1·8199	1·2868	0·9099	0·6434
	37 : 27	6·3214	4·4699	3·1607	1·9990	1·4135	0·9995	0·7068
	36 : 28	6·3498	4·4900	3·1749	2·0080	1·4199	1·0040	0·7100

Bezeichnenderweise sind dann stets die Kombinationen der Parentalgeneration die bevorzugten, während die Neukombinationen in geringerer Frequenz auftreten.

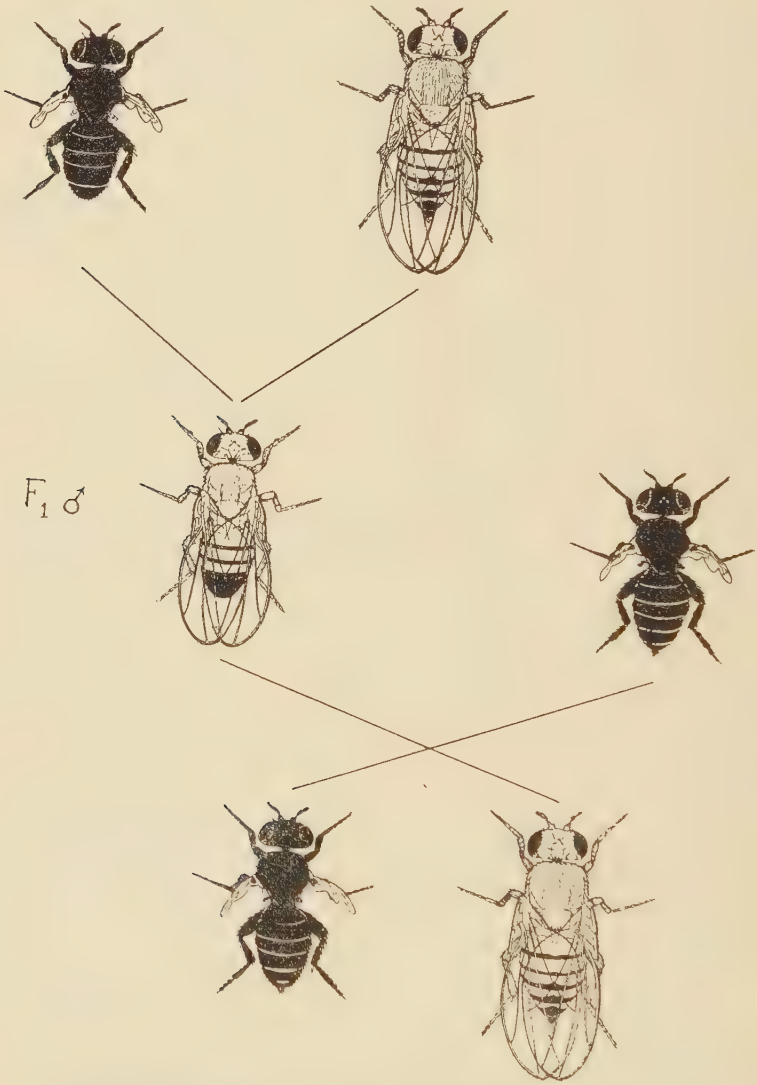


Fig. 60. Graphische Darstellung des Vererbungsganges von vier gekoppelten recessiven Merkmalen (schwarze Körperfarbe, purpur Augen, Stummelflügel und gefleckte Flügelbasis). Das F_1-Männchen wird rückgekreuzt mit einem Weibchen der recessiven Rasse. In der F_2 erscheinen ausschließlich die zwei großelterlichen Kombinationen.

(Nach *Morgan*.)

Dieses charakteristische Zusammenbleiben der Gene in den durch die Gameten der Ausgangsformen eingeführten Kombinationen wird als K o p p e l u n g (*Linkage*, *Morgan*, 1911) bezeichnet. In Fig. 60 ist ein Fall extremer (absoluter) Koppelung dargestellt. Das Drosophila *P*-Männchen ist homozygoter Träger der vier recessiven Merkmale: schwarz (Körperfarbe), purpurn (Augenfarbe), stummelflügelig und fleckig (an der Flügelbasis!). Das *P*-Weibchen ist von normaler Wildform, also Träger der vier dominanten Allelomorphs. Nach der Rückkreuzung des F_1-Männchens mit einem Weibchen der recessiven *P*-Rasse würden bei freier Kombination der vier Merkmale $2^4 = 16$ verschiedene Typen je zu gleichen Teilen zu erwarten sein. Tatsächlich erscheinen aber nur die beiden *P*-Formen, und zwar in der Proportion 1 : 1. Die Kombinationen der vier recessiven Merkmale — bzw. der vier dominanten Allelomorphs — sind demnach erhalten geblieben. Diese Eigenschaften werden nicht f r e i m e n d e l n d sondern g e k o p p e l t vererbt.

Koppelungsgruppen sind indessen nur selten wie hier a b s o l u t g e k o p p e l t. Bisher ist eine solche Stabilität hauptsächlich bei Drosophilamännchen an großem Material zweifelsfrei nachgewiesen. Dagegen können bei der Gametenbildung der Drosophilaweibchen einzelne Glieder der Eigenschaftsgruppen wechselweise ausgetauscht werden. Der Vorgang wurde von *T. H. Morgan* 1911 als C r o s s i n g - o v e r beschrieben; unter den deutschen Genetikern hat sich die Bezeichnung F a k t o r e n a u s t a u s c h eingebürgert. In Rückkreuzungen von Bastardweibchen mit Männchen der totalrezessiven *P*-Rasse treten darum auch die Neukombinationen gekuppelter Eigenschaften auf (in Fig. 61 weiße Augen und lange Flügel bzw. rote Augen und kurze Flügel). Ihre Häufigkeit wechselt (immer nur je zwei Merkmalspaare in Rechnung gestellt) zwischen 0 und 50%. Im dargestellten Beispiel machen sie 33% der Rückkreuzungsgeneration aus. Es ist evident, daß bei 50% Neukombinationen der Effekt der freien Kombination erreicht wird.

Die Neukombinationen durch Austausch zwischen Koppelungsgruppen müssen wohl unterschieden werden von denen, welche aus der freien Kombination nach den *Mendel*schen Regeln gebildet werden. Denn die Prüfung der weiteren Generationen zeigt, daß die Austauschneukombinationen wiederum gekoppelt sind und in der neuen Verbindung genau die gleiche Festigkeit besitzen — oder, was dasselbe heißt, den gleichen Austauschwert haben — wie die Ausgangskombinationen. Im Interesse der Klarheit der Terminologie empfiehlt es sich, auch in der deutschen Sprache für die Austauschneukombinationen *Morgans* Bezeichnung R e k o m b i n a t i o n zu verwenden.

Während, wie erwähnt, bei den verschiedenen Drosophilaspezies der Faktorenaustausch nur in der Ovogenese vor sich geht, hat man einen solchen bei manchen Pflanzen, Fischen und Nagern in beiden Geschlechtern nachgewiesen. Bei Ratten und Mäusen

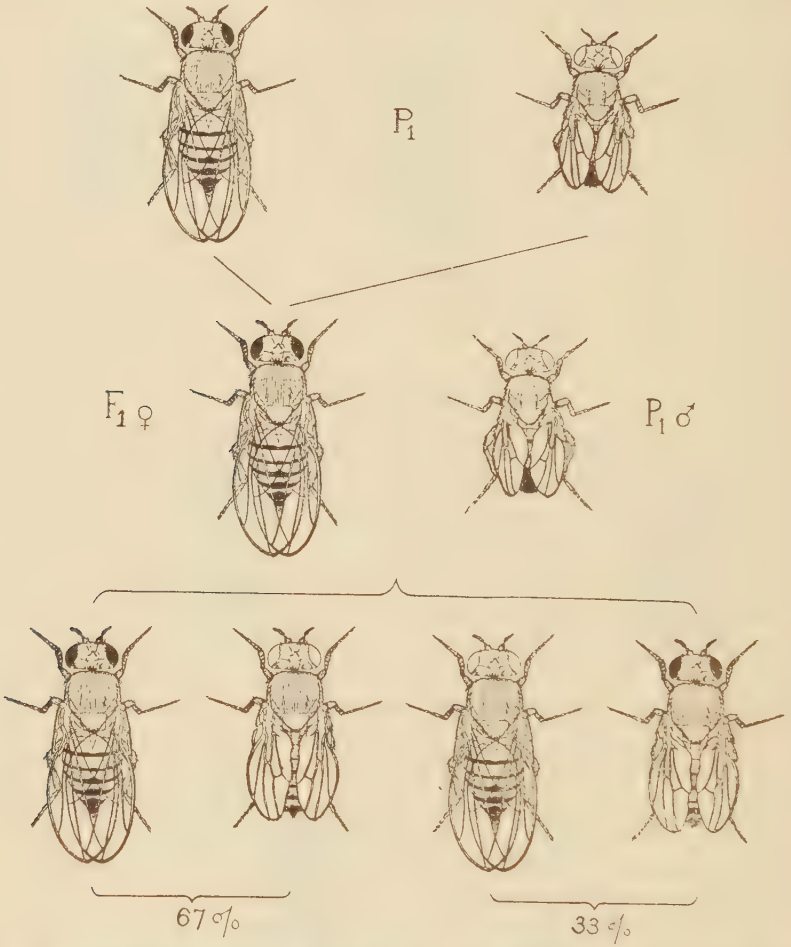


Fig. 61. Vererbungsgang von zwei gekoppelten recessiven Merkmalen (weiße Augen und Miniaturflügel). Die Anordnung des Experimentes ist von dem der vorigen Abbildung dadurch verschieden, daß ein Weibchen der F_1 und ein Männchen der recessiven Rasse zur Weiterzucht verwendet werden. In F_2 finden sich die großelterlichen Kombinationen nur mit 67%. Die restlichen 33% zeigen „Rekombination" der Merkmale. (Nach *Morgan*.)

scheint der Prozentsatz bei den Weibchen stets höher zu sein als bei den Männchen (*Castle*, 1925). Diese Differenz läßt vermuten, daß die absolute Koppelung in den Drosophilamännchen aus einer fortschreitenden Herabsetzung der männlichen Austauschrate resultierte.

Wir dürfen heute schon aus guten Gründen annehmen, daß eine jede Spezies eine charakteristische Anzahl von Koppelungsgruppen besitzt, obschon diese Zahl gegenwärtig nur in wenigen Fällen durch Vererbungsexperimente bestimmt worden ist. Es versteht sich, daß Merkmale, die verschiedenen Kupplungsgruppen angehören, sich nach den Regeln der freien Kombination verbinden, und alle unsere Erörterungen unter dem Paragraphen C (S. 522 bis 536) haben darum ausschließlich Gültigkeit in bezug auf Faktoren, welche in verschiedenen Koppelungsgruppen liegen.

T. H. Morgan hat die Koppelungserscheinung auf die Lokalisation der Faktoren im selben Chromosom zurückgeführt. Die grundlegende Tatsache zugunsten dieser Hypothese ist in der Indentität der Zahl der Koppelungsgruppen mit der haploiden Chromosomenzahl gegeben. Diese Identität wurde in allen Spezies gefunden, die genetisch gut durchgearbeitet sind. In anderen Spezies mit einer geringeren Zahl bekannter Erbfaktoren (und eventuell höherer Zahl von Chromosomen!) ist die Zahl der bekannten Gruppen stets kleiner als die der Chromosomen. Die Hypothese der chromosomalen Lokalisation ist aber insbesondere durch parallele genetische und cytologische Studien über geschlechtsgekoppelte Erbfaktoren und die besonderen Vererbungserscheinungen bei abnormen Chromosomenkombinationen in einem Grade von Wahrscheinlichkeit als richtig erwiesen worden, der praktisch mit Sicherheit bezeichnet werden muß. Kein Versuch, die Koppelungserscheinungen auf ein anderes Prinzip zurückzuführen, hat irgendwelchen Erfolg gehabt.

Das Phänomen des Faktorenaustausches muß dann konsequenterweise auf einen Austausch entsprechender Teile homologer Chromosomen zurückgeführt werden. Auf diese Basis bezogen zwingen die Besonderheiten des Vorganges zur Postulierung einer linearen Anordnung der Gene in den Chromosomen. (Vgl. außer *Morgans* Schriften auch die kritische Prüfung durch *Jennings* 1923).

Aus diesen einleitenden Erörterungen geht hervor, daß es möglich sein muß, Erbfaktoren zu lokalisieren

1. durch die Bestimmung der Koppelungsgruppe, welcher sie zugehören;
2. durch die Bestimmung des Ortes (Locus) innerhalb der Reihenfolge dieser Gruppe.

Bestimmung der Koppelungsgruppe.

Koppelungsgruppen werden provisorisch in der Reihenfolge, wie sie entdeckt werden, fortlaufend numeriert. Sofern beim Fortschritt der Studien in verschiedenen Spezies homologe Chromosomen bzw. Koppelungsgruppen) nachgewiesen werden können,

mögen sie wohl mit gleichen Nummern bezeichnet werden. So wird die geschlechtsgekoppelte Gruppe im Anschluß an *Morgan* stets als die erste (I) bezeichnet.

Die Zugehörigkeit zur ersten Gruppe kann demnach leicht aus den Besonderheiten der geschlechtsgekoppelten Vererbungsweise erkannt werden, welche im nächsten Abschnitt behandelt werden.

Die autosomalen Gruppen sind unter Umständen nicht leicht zu erkennen. Wenn — wie bei Drosophila — der Austausch stets nur in der Ovogenese statthat, dann ist die Sachlage noch günstig. Angenommen, die eine Rasse besitze beide recessiven (*ab*) die andere dagegen die beiden dominanten Merkmale (*AB*), dann erhalten wir in F_2 entweder das Verhältnis der freien Kombination

$$9\ AB : 3\ Ab : 3\ aB : 1\ ab,$$

was fast immer bedeuten würde, daß die Faktoren verschiedenen Koppelungsgruppen zugehören oder aber die Rekombinationen *Ab* und *aB* sind seltener und falls die Abweichung bedeutend ist, kann auf Koppelung der beiden Faktoren geschlossen werden. Je mehr das Resultat sich dem Verhältnis

$$3\ AB : 1\ ab$$

nähert (das absolute Koppelung in beiden Geschlechtern bedeuten würde!), desto enger ist die Koppelung, z. B. würde eine Austauschrate von 10% eine Nachkommenschaft in der Zusammensetzung von

$$29\ AB : 1\ Ab : 1\ aB : 9\ ab$$

ergeben.

Weit häufiger wird es aber vorkommen, daß jede Rasse je ein dominantes und ein recessives Merkmal besitzt, also *Ab* bzw. *aB*. Dann zeigt die F_2 ohne Koppelung wieder das Verhältnis der freien Kombination, mit Koppelung jedoch unter allen Umständen die charakteristische Proportion

$$2\ AB : 1\ Ab : 1\ aB : 0\ ab.$$

In der Drosophilaarbeit, wo nahezu alle autosomalen Faktoren entweder im zweiten oder im dritten Chromosom liegen, wird jetzt meistens eine andere Methode verwendet. Fliegen mit einer neuen recessiven Mutation werden mit einer Rasse gekreuzt, die im zweiten Chromosom den dominanten Faktor Star und im dritten Chromosom den dominanten Faktor Dichaete führt. Männchen der F_1 werden dann rückgekreuzt mit Weibchen der neuen Mutante. Da in den Bastardmännchen kein Austausch stattfindet, so wird einer der folgenden Fälle realisiert werden. 1. Keine Dichaete der Rückkreuzung zeigen den neuen Charakter, wohl aber ist das der Fall

bei der Hälfte der Starfliegen[1]). Das bedeutet Zugehörigkeit zur Gruppe III. 2. Keine Starrückkreuzungstiere zeigen den neuen Charakter, dagegen kombiniert er sich mit der Hälfte der Dichaete[1]). Das erweist Zugehörigkeit zur Gruppe II. 3. In seltenen Fällen mag es vorkommen, daß der neue Charakter sich mit Star sowohl als mit Dichaete frei kombiniert. Das würde Lokalisation in der IV. Gruppe anzeigen.

In Spezies mit Austausch in beiden Geschlechtern sind die Verhältnisse entsprechend kompliziert. Stets kommt die Koppelung jedoch in Abweichungen von der 9 : 3 : 3 : 1-Proportion in F_2 zum Ausdruck, und zwar im Sinne einer geringeren Frequenz der beiden Rekombinationsklassen. Ob wirklich Koppelung vorliegt, kann dann weiterhin durch Rückkreuzungen mit Totalrecessiven geprüft werden. Letzterer Versuch führt gleichzeitig zur Ortsbestimmung.

Bestimmung des Ortes eines Erbfaktors innerhalb seiner Koppelungsgruppe.

Die Drosophilastudien haben klar demonstriert, daß nicht einzelne Faktoren aus den Koppelungsgruppen losgelöst und aus-

Fig. 62. Schema der linearen Anordnung der Gene.

getauscht werden, sondern relativ große Teile der ganzen Serie. Ob die Bruch- bzw. Wiedervereinigungsstelle stets zwischen zwei Faktoren liegt, ist heute mit Bestimmtheit wohl nicht entschieden. Jedenfalls aber entspricht das dem Verhalten der Regel nach. Rein heuristisch können wir uns daher eine Koppelungsgruppe vorstellen als eine Reihe einzelner Faktoren und zwischenliegender Bruchstellen (Fig. 62). Daß alle Glieder dieser Kette die gleiche metrische Ausdehnung haben und daß der Austausch an sämtlichen Bruchstellen mit gleicher Leichtigkeit sich vollziehe, ist unwahrscheinlich. So viel ist aber trotzdem leicht ersichtlich, daß zwei Faktoren einer Serie im allgemeinen um so häufiger durch Austausch voneinander getrennt werden müssen, je mehr Glieder zwischen ihnen liegen. Ausgehend von dieser Erwägung hat *T. H. Morgan* das Austauschprozent als Maß der Entfernung gewählt. Viel Mißverständnis und Widerspruch gegenüber *Morgans* Werk beruht darauf, daß eine solche Einheit irrtümlich metrisch — etwa als ein bestimmter Bruchteil eines Mikromillimeters — auf-

[1]) Wenn zur Rückkreuzung nicht homozygote Weibchen der Mutante, sondern Bastarde mit der Normalform verwendet werden, dann ist hier zu lesen „bei einem Viertel der Star" bzw. „bei einem Viertel der Dichaete".

gefaßt wird, während sie lediglich derjenigen Strecke entsprechen kann, innerhalb deren Grenzen in durchschnittlich 100 Generationsfolgen ein Bruch mit folgendem Austausch statthat. Diese Einheit muß demnach in ihrer metrischen Längenausdehnung der Koppelungsfestigkeit direkt proportional sein. Nun erweist sich aber die Koppelungsfestigkeit als veränderlich. Bei Drosophila wechselt sie individuell mit dem Alter der Tiere (*Bridges*, *Plough*), mit der Temperatur (*Plough*), unter dem Einfluß von Röntgenbestrahlung (*Mavor*, *Muller*), und ebenso sind erbliche Varianten häufig festgestellt und in einigen Fällen durch Selektion isoliert worden (*Sturtevant*, *Bridges*, *Morgan*, *Detlefsen*, *Just*). Bei konstanten Lebensbedingungen und weitgehendster genetischer Identität des Zuchtmaterials fand *Just* in der ersten Koppelungsgruppe von Drosophila nahezu konstante Austauschwerte, während *Gowen* unter ähnlichen Bedingungen im dritten Chromosom immer noch eine hohe Variabilität feststellte. Auch innerhalb desselben Chromosoms sind Unterschiede festgestellt, da die Austauschrate in der Mitte des zweiten und dritten Chromosoms stärker variiert als in den „subzentralen" und distalen Abschnitten (*Muller*). Wie bereits erwähnt, übt auch das Geschlecht einen Einfluß aus (*Castle*, *Dunn*, *Wachter*, *Detlefsen*).

Der von *Morgan* oft für den Austauschwert gebrauchte Ausdruck „Mapdistance" muß nach alledem in durchaus metaphorischem Sinne verstanden werden. Wörtlich kann er höchstens auf die graphische Darstellung der Austauschwerte in den Chromosomenkarten bezogen werden. Als wesentlichstes Ergebnis all dieser Studien muß jedoch hervorgehoben werden, daß trotz der erwähnten Variabilität nie die Reihenfolge der Merkmale verändert erscheint, und das ist natürlich für die Theorie der linearen Anordnung der Gene in den Chromosomen von entscheidender Bedeutung. Im übrigen erhellt aus diesen Tatsachen wiederum die Notwendigkeit, genetische Experimente unter möglichst strenger Kontrolle aller physiologischen Bedingungen durchzuführen. Auch hier sind in der Regel optimale Lebensbedingungen anzustreben.

Wenn nach der oben dargelegten Methode die Zugehörigkeit einer neuen Eigenschaft zu einer bestimmten Koppelungsgruppe festgestellt ist, so kann unmittelbar die Bestimmung des Ortes in der linearen Reihe der Faktoren in Angriff genommen werden. Von einer solchen Ortsbestimmung kann erst die Rede sein, wenn die Gruppe aus wenigstens drei Faktoren besteht. Nehmen wir im Anschluß an ein von *Morgan* ausführlich dargestelltes Beispiel an, es seien aus der dritten Gruppe der Drosophila melanogaster bereits bekannt die beiden dominanten Mutationen Dichaete (*D*) und Hairless (*H*), und es werde neu gefunden die Mutation hairy (*h*). Zunächst wird nun die F_1 aus $DH \times h$ hergestellt. Dann werden die

F_1-Weibchen rückgekreuzt mit den Doppeltrecessiven, d. h. mit der homozygoten hairy-Rasse. Die Nachkommen ohne Austausch sind dann je zur Hälfte *DH* bzw. *h*. Sie machen in einem gewissen Experiment 54% der Nachkommenschaft aus, während der Rest sich aus den verschiedenen Rekombinationsklassen zusammensetzt. Wenn der Austausch irgendwo zwischen *D* und *H* stattfindet, so entstehen die beiden Rekombinationen $+ H$ und $D +$, und zwar erwartungsgemäß in ungefähr gleicher Zahl (in diesem Experiment 142 bzw. 143 Individuen). Sie machen zusammen als Dichaete-Hairless-Klasse 32% der Rückkreuzungsgeneration aus. In gleicher Weise wurden die hairy-Hairless-Klasse mit 40% und die hairy-Dichaete-Klasse mit 18% vertreten gefunden. Da es sich um ein Dreipunktexperiment handelt, so zählt jedes Individuum in zwei verschiedenen Klassen mit. Alle Austauschindividuen zusammen genommen machen daher doch nur 45% der Gesamtnachkommenschaft aus. Aus diesen Daten folgt, daß hairy näher bei Dichaete liegt als bei Hairless und daß Dichaete seinen Platz zwischen hairy und

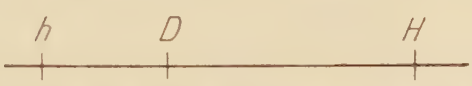


Fig. 63. Relative Position von drei Faktoren in der dritten Koppelungsgruppe.

Hairless haben muß. Die gegensätzlichen Richtungen in der Chromosomenkarte werden von *Morgan* mit links und rechts bezeichnet, so daß hairy links und Hairless rechts von Dichaete lokalisiert erscheinen (vgl. Fig. 63).

Bei 18% Austausch zwischen *h* und *D* und 32% zwischen *D* und *H* berechnet sich der Austausch zwischen *h* und *H* mit 50%. Oben fanden wir für die hairy-Hairless-Klasse nur 40%. Wenn wir nämlich nur diese beiden Merkmale in Betracht ziehen, so müssen die 10% Austausch unserer Beobachtung entgehen, welche auf den Doppelaustausch entfallen. Es ist klar, daß alle die Tiere, welche Austausch im Intervall *hD* sowohl als auch zwischen *D* und *H* erfahren haben, in jeder der ersten zwei Klassen mitgezählt wurden, dagegen bei der hairy-Hairless-Klasse ausfielen, weil in bezug auf diese beiden Merkmale die Ausgangsverhältnisse wiederhergestellt erscheinen. Diese Klasse mit doppeltem Austausch ist stets die kleinste; im hier analysierten Falle umfaßt sie 5% der Rückkreuzungsgeneration. Es ergibt sich daraus die Tatsache von fundamentaler Bedeutung, daß die Zahl der Rekombinationen eventuell kleiner ist als die der Austausche. Da nur die ersteren direkt in Erscheinung treten, die Chromosomenkarten jedoch auf dem Austauschprozent

basieren, so müssen bei der Auswertung der Rekombinationsprozente entsprechende Korrekturen angebracht werden.

Die Erfahrung hat nun gezeigt, daß allerdings — wie zu erwarten — im allgemeinen der Rekombinationsverlust durch doppelten Austausch mit der Distanz wächst bzw. sich vermindert. Aber gleich bei seiner ersten Studie über doppelten Austausch bemerkte *Sturtevant* (1913), daß hier keine einfache Proportionalität herrscht. Es hat sich gezeigt, daß von einer Austauschstelle stets eine gewisse Schutzwirkung auf die benachbarten Orte ausgeht, so daß in nächster Nähe überhaupt kein zweiter Bruch stattfindet und mit steigender Entfernung erst allmählich diejenige Häufigkeit erreicht wird, welche der Erwartung entsprechen würde. Nehmen wir, um das klarzumachen, an, ein Abschnitt *AB* einer Koppelungsgruppe zeige 8% Austausch, ein nahe dabei liegender Abschnitt *CD* 10% und ein entfernt liegender Abschnitt *MN* ebenfalls 10%. Wenn *AB* und *MN* gleichzeitig untersucht werden, so ist erwartungsgemäß gleichzeitiger Austausch in beiden Abschnitten in 10 von 8% oder in 0·8% der Individuen zu beobachten. Dagegen sind die Fälle von Doppelaustausch bei gleichzeitiger Betrachtung von *AB* und *CD* wesentlich seltener, sagen wir 0·2%. Das Verhältnis der Doppelbrüche, welche tatsächlich beobachtet werden, zu denen, welche unter der Voraussetzung, daß die Austausche in zwei beliebigen Regionen stets unabhängig voneinander erfolgen, erwartet würden, bezeichnet *Morgan* als **Koinzidenz**. In unserem ersten Beispiel wäre die Koinzidenz $\frac{0{\cdot}8}{0{\cdot}8} = 1$; im zweiten Falle $\frac{0{\cdot}2}{0{\cdot}8} = 0{\cdot}25$. Im oben analysierten hairy-Dichaete-Hairless-Experiment fanden sich 5% Doppelaustauschindividuen. Zu erwarten wären 6%, so daß die Koinzidenz ungefähr 0·8% beträgt. (Es sei bemerkt, daß alle hier verwendeten Daten später wesentlich korrigiert wurden, was jedoch die methodische Seite nicht weiter berührt.) Auf eine Entfernung von wenigstens fünf Einheiten links und rechts einer Bruchstelle ist der sekundäre Austausch (und damit die Koinzidenz in diesem Abschnitt) gleich Null. Das Phänomen der teilweisen oder völligen Verhinderung eines sekundären Austausches durch die primäre Bruchstelle wird als Interferenz bezeichnet. Die Kurven der Fig. 64 geben eine graphische Darstellung des Rekombinationsverlustes. Ihr Verlauf ist bestimmt durch die zwei Faktoren Doppelaustausch und Interferenz. *Morgan* heißt sie kurz Korrektionskurven, da ihre Ordinaten die Werte darstellen, welche zu den experimentell ermittelten Rekombinationswerten addiert werden müssen, um die korrigierten Austauschwerte zu erhalten.

Das Verhältnis von Rekombination und Austausch erhellt darum aus den Kurven der Fig. 65. Die Rekombinationskurven streben alle der 50%-Grenze zu. Das bedeutet nichts anderes, als daß Faktoren, die weit genug auseinanderliegen, sich nach den Zufallsregeln kombinieren (genau wie solche, welche verschiedenen Koppelungsgruppen zugehören). Dieser Zustand ist für roughoid (*ru*)-Hairless nahezu erreicht. Experimentell wurden 45·2% Rekombinationsfälle gefunden; die ausgeglichene Kurve führt sogar auf 49·1%. Die *ru*-Kurve der Fig. 64 zeigt auf der *H*-Ordinate einen Rekombinationsverlust von 20·4. Der korrigierte Austauschwert berechnet sich demnach als 69·5.

Es ist selbstverständlich, daß die Zugehörigkeit zur gleichen Koppelungsgruppe für Merkmale, wie roughoid und Minute-g

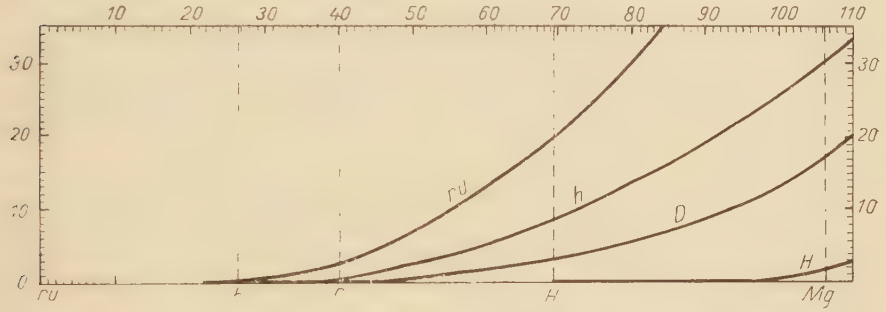


Fig. 64. Korrektionskurven für vier Faktoren der dritten Koppelungsgruppe. Die Ordinaten stellen den Rekombinationsverlust dar.
(Nach *Morgan*.)

(*Mg*), nur aus ihrer Koppelung mit zwischenliegenden Faktoren gefolgert werden kann.

Die beiden Kurvendarstellungen lassen erkennen, daß die Interferenz sich in der Mitte der Koppelungsgruppe schwächer bemerkbar macht als nach den Enden zu. Rechts von Dichaete (*D*) finden wir den Austausch nur auf eine Distanz von zirka fünf Einheiten völlig unterdrückt, während für roughoid (*ru*) die entsprechende Distanz ungefähr 25 Einheiten mißt.

Da die Chromosomenkarten auf den Austauschwerten basieren, so sollten bei Ortsbestimmungen die Basisfaktoren nicht zu weit auseinanderliegen, so daß die Korrekturen möglichst niedrig bleiben. Als Regel kann gelten, daß sie nicht weiter als 30 Einheiten auseinander sein sollten. Im oben analysierten Beispiel wurde der neue Faktor (*h*) außerhalb der bekannten (*D H*) liegend gefunden. Günstiger ist es, wenn der neue Faktor von den bekannten links und rechts flankiert wird.

Die Konstruktion der Chromosomenkarten.

Eine der Hauptschwierigkeiten bei der Konstruktion der Chromosomenkarten beruht auf dem Umstand, daß als Maßeinheit das Austauschprozent verwendet werden muß. Alle sekundären Bestimmungen sind damit abhängig von der Basisbestimmung, auf der sie beruhen. Wenn z. B. *D* als Ausgangspunkt gewählt und darauf die folgenden Bestimmungen *H*, *ca*, *bn* usw. bezogen werden, dann muß natürlich jede spätere Korrektur irgendeiner dieser Lokalisationen die Werte aller nachfolgenden verschieben. Heute steht in der dritten Koppelungsgruppe von Drosophila roughoid

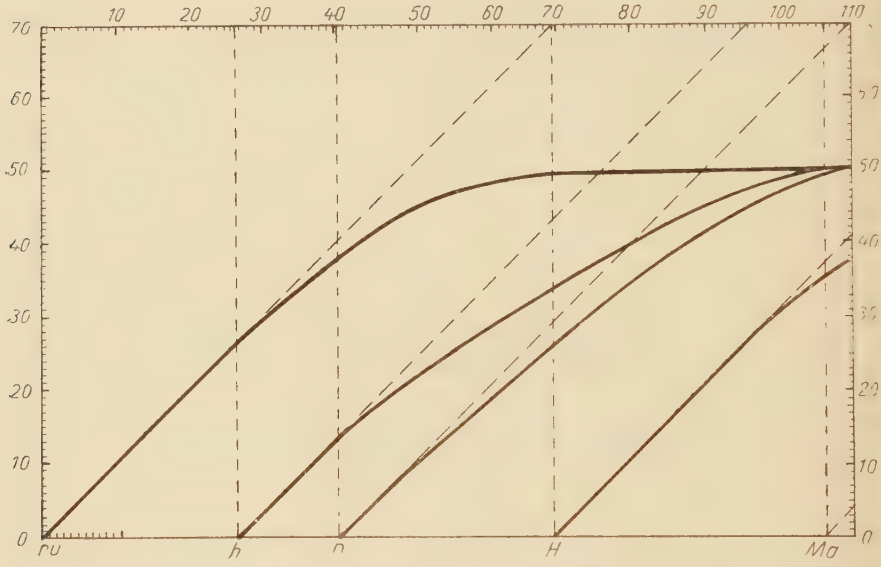


Fig. 65. Rekombinationskurven von fünf Faktoren der dritten Koppelungsgruppe. (Nach *Morgan*.)

am linken Ende der Reihe. Es muß aber stets mit der Möglichkeit gerechnet werden, daß noch Faktoren weiter links existieren und sich eines Tages durch Mutation zu erkennen geben werden. Das würde wiederum eine völlige Umarbeitung der Chromosomenkarte bedingen, sofern man nicht zur Einführung von Minuswerten übergehen will. Es ist klar, daß bei solchen Verhältnissen jede Chromosomenkarte zuerst sehr unvollständig und skizzenhaft sein muß. Mit fortschreitender Äufnung von Austauschdaten wird sie korrigiert oder, so oft das geboten erscheint, vollständig neu konstruiert.

Bei jeder Neukonstruktion werden die sämtlichen älteren und neueren Experimentalergebnisse verwendet, sofern sie nicht sichtlich durch erbliche oder physiologische Modifikatoren abgeändert sind.

Für jede Klasse wird dann aus der Summe aller Individuen und der Summe der Rekombinationen das Rekombinationsprozent berechnet. Bevor solche Prozente für die Konstruktion der Karte verwendet werden, müssen sie soweit wie möglich korrigiert werden, und zwar durch Addition des Prozentsatzes der in den Rekombinationen nicht gezählten Austausche, d. i. des Verlustes durch Doppelaustausch. Das einzige Mittel, das zur Entdeckung dieses Verlustes führen kann, ist die Zerfällung der Strecke in kleinere Abschnitte. So wurden in sepia (*se*) - rough (*ro*) Austauschkreuzungen aus einem Total von 21.639 Individuen 42·8% Rekombination gefunden. Ein so hohes Prozent kennzeichnet eine relativ große Distanz und dementsprechend ist mit einem großen Verlust von vornherein zu rechnen. Eine Fünfpunktkreuzung hat einen beträchtlichen Teil desselben zum Vorschein gebracht. Die drei zwischengeschalteten Punkte waren Dichaete (*D*), spineless (*ss*) und sooty (*e*). Diese fünf Punkte begrenzen vier Abschnitte (Fig. 66). Doppelaustausche innerhalb eines einzelnen derselben müssen natürlich auch jetzt noch unentdeckt bleiben. Alle Doppel-

Fig. 66. Lokalisation von fünf Faktoren in der dritten Koppelungsgruppe.

austausche jedoch mit Bruchstellen in verschiedenen Abschnitten werden leicht erkannt. Dieses Experiment umfaßt total 1881 Individuen. Die Tabelle 7 gibt eine Übersicht über die gefundenen Doppelaustausche. Die drei letzten Kolonnen enthalten sogar einige Fälle von dreifachem Austausch. Es ist aber klar, daß sie beim Auszählen der *se-ro*-Rekombinationen schon mitgezählt wurden, jedoch nur so, als ob ein einziger Bruch stattgehabt hätte. Die zwei weiteren Bruchstellen müssen daher jetzt noch in Rechnung gestellt werden, d. h. die Fälle dreifachen Austausches sind in die Korrektur als Doppelaustausche einzukalkulieren. So zählen wir denn total 150 Doppelaustausche oder 300 einfache Austausche, die bei der *se-ro*-Bestimmung entgangen waren. Bezogen auf die 1881 Individuen dieses Experiments bedeutet das einen nachgewiesenen Rekombinationsverlust von 16%. Dieser Betrag muß also zu den früher ermittelten 42·8% hinzugezählt werden, so daß wir für *se-ro* ein korrigiertes Austauschprozent von 58·8 erhalten. Auch dieser Wert ist noch zu niedrig. Bei weiterer Zerlegung der vier Abschnitte werden noch mehr Doppelaustausche gefunden, so daß der Abstand *se-ro* in der Chromosomenkarte von 1926 nicht weniger als 65·1 Einheiten mißt (vgl. Fig. 56, S. 512).

TABELLE 7.

Brüche in den Abschnitten . .	1—2	1—3	1—4	2—3	2—4
Anzahl der Individuen	19	33	33	12	37

Brüche in den Abschnitten . .	3—4	1—2—3	1—2—4	1—3—4
Anzahl der Individuen	8	2	4	2

Korrekturen werden, wie leicht ersichtlich, in dem Moment endgültige, in dem ihre Berechnung aus Teilabständen erfolgt, die so kurz sind, daß die Interferenz jeden inneren Doppelaustausch ausschließt. Überhaupt ist zu bemerken, daß Chromosomenkarten um so genauer sind, je mehr Daten von kurzen Intervallen hinein verarbeitet wurden.

Die Konstruktion der Karte erfolgt dann zweckmäßig von dem Punkte aus, für den zahlreichste Beobachtungen vorliegen und der mit der größten Zahl verschiedener Faktoren kombiniert wurde. Für das dritte Chromosom der Drosophila wählte *Morgan* aus solchen Gründen D i c h a e t e als erste Bezugsbasis. In langen Kupplungsgruppen werden zweckmäßigerweise gleich mehrere solcher „Ankerpunkte" gewählt. Dieses Verfahren empfiehlt sich besonders auch darum, weil die Faktoren selber nicht gleichmäßig über die Kartenlänge verteilt erscheinen, sondern mehr oder weniger distinkte Gruppen bilden (vgl. Fig. 56). Neben Dichaete wurden deshalb Hairless als sekundäre und claret als tertiäre Basis benutzt. Von diesen Basispunkten aus werden dann die umliegenden Orte bestimmt. Zuerst die am nächsten liegenden. In der Hairlessgruppe ist e b o n y der erste Punkt. Er zeigt mit *H* einen Austauschwert von 1·2. Die Individuenzahl dieser Klasse ist 3992, womit der Wert als weitgehend gesichert gelten darf. Der nächste Faktor ist B a n d. Sein Ort würde sich aus den direkten *H-bn* Experimenten mit 2·4 Einheiten rechts von *H* bestimmen. Es sind hier nur 2370 Individuen für die Bestimmung zur Verwendung gekommen, weshalb eine Verifikation aus anderen Experimenten wünschbar erscheint. Dazu stehen die Daten der Delta-band Kreuzungen zur Verfügung, die bei 281 Individuen den Austauschwert 6·8 zeigen. Nun wurde aber Delta selber noch nicht neu lokalisiert. In der alten Karte steht es 3·3 Einheiten links von *H*, so daß sich darauf gestützt der Lokus von Band mit 3·5 rechts von *H* berechnen würde. Beim Ausgleich zwischen den zwei Bestimmungen muß nun natürlich ein System des Abwägens gefunden werden und *Morgan* spricht auch geradezu vom „G e w i c h t" einer Bestimmung. In allen direkten Experimenten wird die einfache Zahl der beteiligten Individuen als Gewicht eingesetzt. Im Falle Delta-band, wo beide Faktoren neu bestimmt werden sollen, wird nur die halbe Zahl der Individuen jedem

der Orte zugeschrieben. Der Ort für band bestimmt sich demnach als

$$\frac{2{\cdot}4 \,.\, 2370 + 3{\cdot}5 \,.\, 140}{2370 + 140} = 2{\cdot}5$$

Als nächster Faktor folgt nun Delta. Dafür stehen folgende Bestimmungen zur Verfügung.

Delta-*H* 2·9, Gewicht 4469.

Delta-ebony 4·4, konvertiert Delta-*H* 3·2, Gewicht 861.

Delta-band 6·8, konvertiert Delta-*H* 4·3, Gewicht 140 (halbe Individuenzahl).

Delta-white ocelli 10·3, konvertiert Delta-*H* 3·5, Gewicht 155 (halbe Individuenzahl).

Daraus berechnet sich ein Ort für Delta 3·0 Einheiten links von *H*. (Nach *Bridges* und *Morgan*, 1923, wären es 3·3 Einheiten, doch finde ich für diese Berechnung keine Unterlagen.)

Da einige der Faktoren gegenseitig voneinander abhängig sind, so werden die Berechnungen jedesmal nach Angliederung einer weiteren Serie von Faktoren wiederholt und dabei die zuletzt ermittelten Ortsbestimmungen als Basis genommen. Auf diese Weise können gelegentlich noch geringfügige Verbesserungen erzielt werden.

Nachdem auf solche Weise alle Abstände möglichst genau bestimmt worden sind, wird dann der am weitesten links liegende Faktor der Koppelungsgruppe als N u l l p u n k t zum Ausgangsort für die endgültige Redaktion der Chromosomenkarte gemacht und auf ihn werden nun in einfacher Weise alle die anderen Werte bezogen.

Die Austauschunterdrücker und ihre Verwendung in der Herstellung absolut homozygoter Individuen.

Für manche genetische Studien ist es von Wichtigkeit, mit Material arbeiten zu können, das absolut homozygot ist, bei dem also keine hereditären Unterschiede bezüglich der unkontrollierten (und unkontrollierbaren) Modifikatoren und kleinen Mutanten bestehen. Solche „reinen Linien" erhalten bekanntlich die Botaniker durch engste Inzucht, nämlich durch die Propagation vermittels Selbstbestäubung. Für getrenntgeschlechtige Tiere tritt an deren Stelle die mühsame Selektionszucht, die jedoch niemals ebenbürtige Resultate zu liefern vermag. Neulich haben nun die Drosophilaforscher (namentlich *Bridges*) eine Methode ausgearbeitet, die es erlaubt, innerhalb von zwei Generationen absolute Homozygotie bezüglich der drei ersten Chromosomen herzustellen.

Das gelingt mit Hilfe der Austauschunterdrücker (Crossing-over suppressor), die im Laufe der letzten Jahre aufgefunden worden sind. Diese scheinen cytologisch in Chromosomensplittern zu bestehen, die sich an intakten Chromosomen festgesetzt haben und nun den Austausch für eine größere oder kleinere Distanz verhindern. Wenn in einem Chromosom der gesamte Austausch unterdrückt ist, dann kann die homozygote Sippe hergestellt werden, wie das mit dem folgenden Beispiel für das zweite Chromosom illustriert sei.

Ein Männchen, welches heterozygot für das Merkmal Lobe ist, wird mit einem Curlyweibchen gekreuzt. (Curly ist ein dominanter Charakter, welcher mit Austauschunterdrückern für den linken wie den rechten Schenkel des zweiten Chromosoms assoziiert ist.) Die F_1 besteht demnach aus gleichen Teilen von Curly-Normal und Curly-Lobe. Curly-Normal unter sich weiter gezüchtet gibt in F_2 Curly und Normal im Verhältnis von 3:1. Die letztere Gruppe repräsentiert die gesuchten absolut Homozygoten des zweiten Chromosoms.

E. Der Vererbungsmodus geschlechtsbestimmender und geschlechtsgekoppelter Faktoren.

Der hier zu beschreibende Vererbungsmodus ist zwar vom Schweizer Arzt *Horner* schon im Jahre 1876, also vor der Begründung der Vererbungszytologie beschrieben worden. Aber erst durch die späteren Chromosomenstudien ist er seinem Wesen nach verständlich geworden. Es ist heute allgemein bekannt, daß bei gewissen Tierspezies die Chromosomen in Autosomen und Geschlechtschromosomen (*X*-Chromosomen) geschieden werden können. Die ersteren sind dann in beiden Geschlechtern typischerweise in gleicher Zahl zu finden, und zwar in diploider Anordnung. Demgegenüber sind die Geschlechtschromosomen bloß in einem Geschlecht paarweise, im anderen dagegen haploid vorhanden (Fig. 67). Bei Schmetterlingen und Vögeln ist die diploide Zahl von *X*-Chromosomen bei Männchen, die haploide bei Weibchen gefunden worden. In allen übrigen Fällen ist das Verhältnis gerade umgekehrt. Halten wir uns im folgenden an den letzteren Typus, wie er durch *Protenor* illustriert wird. In den Reifeteilungen bildet das Weibchen natürlich ausschließlich Eier, die den haploiden Satz der Autosomen und ein *X*-Chromosom enthalten. Das Männchen jedoch bildet zwei Spermiensorten. Die eine Hälfte stimmt im Chromosomengehalt mit den Eiern überein und wird deshalb von *Witschi* als Gynospermien bezeichnet. Die andere Hälfte hat nur den Satz der Autosomen, aber kein Geschlechtschromosom; sie werden von den ersteren als Androspermien unter-

schieden. Es ist klar, daß in allen diesen Fällen alle in *X*-Chromosomen lokalisierten Faktoren nach dem Hymenopterentypus (vgl. oben Haplontenmethode!) vererbt werden. Da die Gynospermien mit normalen Eiern Weibchen und die Androspermien im entsprechenden Falle Männchen erzeugen, so vererbt der Vater das *X*-Chromosom ausschließlich auf die Töchter. Dagegen erhalten die Söhne ihr einziges *X* stets von der Mutter.

Da so im normalen Ablauf der Dinge die Gynospermien weibchenbestimmend sind, so liegt nahe, dem *X*-Chromosom einen weibchendeterminierenden Erbfaktor zuzuschreiben (*F*). Korrespondierend muß dann vorausgesetzt werden, daß im Autosomensatz der Androspermien ein männchendeterminierender Faktor (*M*) lokalisiert sei. Bei konsequenter Durchführung dieser Annahmen

Fig. 67. Chromosomen von Protenor belfragei.
a = Anaphase der zweiten Spermatocytenteilung; *b* = Tochterplatten derselben; *c* = Äquatorialplatte einer Spermatogonie; *d* = Äquatorialplatte aus dem Ovarium. *X* = Geschlechtschromosom.
(Nach *Wilson* aus *Schleip.*)

ergeben sich die Geschlechtserbformeln *FFMM* für Weibchen und *FMM* für Männchen. Beide Geschlechter enthalten demnach beide Sorten von Geschlechtsdeterminatoren — das steht in guter Übereinstimmung mit den vielen Beobachtungen über pathologisches und experimentelles Zwittertum — aber in ungleichem Mengenverhältnis. Offensichtlich unterdrücken zwei Weiblichkeitsfaktoren die gleiche Zahl von Männlichkeitsfaktoren, während zwei Männlichkeitsfaktoren stärker sind als ein einzelner Weiblichkeitsfaktor.

Während sonst in erbanalytischen Arbeiten fast ausschließlich das Zusammenwirken von allelomorphen Faktoren studiert wird, so basiert die Analyse der geschlechtsdeterminierenden Faktoren auf einem ganz verschiedenen Prinzip: dem quantitativen Verhältnis entgegengesetzt gerichteter und in verschiedenen

Chromosomen lokalisierter Gene. Wir kommen auf die Methodik der quantitativen Erbanalyse in einem besonderen Kapitel zurück.

Neben dem *XX-X* Typus existiert nun noch der *XX-XY* Typus der Geschlechtschromosomen, der sich nach *Evans* und *Paintner* z. B. beim Menschen realisiert findet. Die Fig. 68 stellt ein hierhergehöriges Beispiel dar. Die Männchen von Lygaeus haben ein *X*-Chromosom, das denen der Weibchen gleichwertig ist, aber außerdem noch einen wenn auch kleineren Partner, der als *Y*-Chromosom bezeichnet wird. Da bei den Reduktionsteilungen das *Y*-Chromosom in die Androspermien geht, so ist es klar, daß es stets vom Vater direkt auf die Söhne vererbt wird, somit aus-

Fig. 68. Chromosomen von Lygaeus turcicus.

a = zweite Spermatocytenteilung; *b* = Tochterplatten derselben; *c* = Äquatorialplatte einer Spermatogonie; *d* = einer Ovogonie; *e* = die Chromosomen von *c* in Paaren; *f* = die Chromosomen von *d* in Paaren. *X* und *Y* sind die Geschlechtschromosomen.

(Nach *Wilson* aus *Schleip.*)

schließlich durch die männliche Linie läuft. Die Fig. 69 zeigt die Vererbungsweisen von *X* und *Y* bei diesem Typus. So müssen denn auch alle eventuell in diesem Chromosom lokalisierten Erbfaktoren einseitig in der männlichen Linie vererbt werden. Nur in wenigen Fällen ist es bisher gelungen, solche Faktoren nachzuweisen. Zuerst (1914) fand *Witschi* bei Fröschen einen geschlechtsbestimmenden Faktor (*f*), der zur Hauptsache für die rassenmäßig bald stärkere, bald geringere Neigung zum Hermaphroditismus verantwortlich ist. Eine dieser Experimentalserien mag hier analysiert werden, da sie methodisch einiges Neue bringt. Es muß vorausgeschickt werden, daß die statistisch am leichtesten erfaßbare

Form des Zwittertums der Frösche im Juvenilhermaphroditismus der Männchen gegeben ist. — Sein Gegenstück, der Adulthermaphroditismus der Weibchen, kommt aus naheliegenden Gründen viel seltener zur Beobachtung. — Während bei bestimmten Rassen (z. B. Davos) die Männchen sich sehr frühzeitig und direkt aus dem indifferenten Zustand heraus differenzieren, findet man bei anderen Rassen (z. B. Freiburg), daß zuerst alle Tiere Ovarien bilden und daß erst während oder nach der Metamorphose die eine Hälfte der Population sich als Männchen zu erkennen gibt, indem ihre Ovarien sich in Hoden umwandeln.

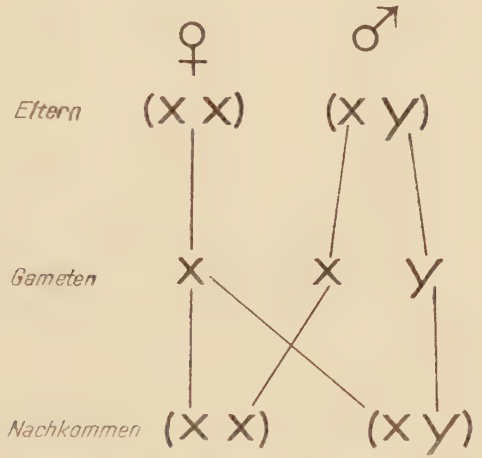


Fig. 69. Vererbungsgang der Geschlechtschromosomen X und Y bei männlicher Heterozygotie.

Wenn also unmittelbar nach der Metamorphose das Geschlechtsverhältnis festgestellt wird, so findet man bei alpinen Rassen 50% Weibchen + 50% Männchen; bei Rassen aus Mitteldeutschland dagegen entweder 100% Weibchen oder einen erhöhten Prozentsatz von Weibchen + Hermaphroditen oder schließlich Weibchen + Hermaphroditen + Männchen in verschiedenen Proportionen (doch nie weniger als 50% Weibchen, wenn die Populationen groß genug sind, um Zufallsresultate auszuschließen).

Nun hat sich in zahlreichen Experimenten erwiesen, daß diese Neigung zum Zwittertum ganz vorwiegend, wenn nicht ausschließlich, durch die Androspermien übertragen wird. Die Methodik, die zur Demonstration der genotypischen Unterschiede zwischen Androspermien verschiedener Rassen führt, ist mit dem Serienexperiment gegeben. (Vgl. zum folgenden die Tabelle 8.)

sperms in zahlreichen Maiskreuzungen (Xenien). Die Zellkerne des Endosperms sind triploid, da sie sich vom diploiden sekundären Embryosackkern herleiten, der vom zweiten generativen Kern des Pollenschlauches befruchtet wurde. Die Matroklinie des Endosperms bedeutet dann, daß zwei Dosen mütterlicher Erbsubstanz über die einfache väterliche Dosis dominieren.

Herbst (1907) und *Landauer* (1922) haben das gleiche Verhältnis bei Seeigelbastarden nachgewiesen. Bastard Plutei aus Sphaerechinus ♀ × Paracentrotus ♂ sind in ihren Merkmalen (Kalkstäbe) intermediär zwischen den Elterformen. Durch geringe Anstöße zur Parthenogenese (Applikation von Kohlensäure oder Ammoniak) können diploide Sphaerechinuseier erzeugt werden, aus denen nach Besamung mit Paracentrotus Sperma sich triploide Larven entwickeln, welche der mütterlichen Form entschieden näherstehen als die diploiden Bastarde.

Im vorangehenden Kapitel haben wir bereits daran erinnert, daß heute allgemein angenommen wird, daß bei gonochoristischen Tieren und Pflanzen entgegengesetzt gerichtete Geschlechtsfaktoren in den Autosomen bzw. Geschlechtschromosomen lokalisiert seien. Eine solche Auffassung basiert notwendigerweise auf der quantitativen Hypothese, daß in einem Fall wie der Drosophilafliege zwei Weiblichkeitsfaktoren stärker seien als zwei Männlichkeitsfaktoren, jedoch ein Weiblichkeitsfaktor schwächer als zwei Männlichkeitsfaktoren.

$$2\,F > 2\,M > F.$$

Diese quantitative Auffassung ist dann durch *Bridges* (1921) und *L. V. Morgans* (1925) Arbeiten mit triploider Drosophilae als wohlbegründet erwiesen worden. Triploide mit einem dreifachen Satz von Autosomen und nur 2-*X*-Chromosomen sind nämlich stets Zwitter. Da sie voraussetzungsgemäß 3-*M*- und 2-*F*-Faktoren führen und hier offensichtlich ein Gleichgewichtszustand erreicht ist, so dürfen wir näherungsweise $3\,M = 2\,F$ setzen, d. h. *F* würde etwa eineinhalbmal so stark sein als *M*, was mit den Grundannahmen gut übereinstimmt.

Triploide Schmetterlinge sind durch *Standfuß*, *Federley* u. a. durch Rückkreuzung von Artbastarden mit den Elternformen hergestellt worden, wobei ähnliche Resultate bezüglich der quantitativen Eigenschaften der Geschlechtsgene erhalten wurden.

Die ausgedehntesten Studien über Polyploidie im Zusammenhang mit Vererbungsanalysen sind von Botanikern ausgeführt worden. Es seien besonders erwähnt *Blakeslees* Experimente mit Stechapfel (Datura) und *v. Wettersteins* Versuche mit Moosen.

Bei Tieren ist es im allgemeinen schwieriger, polyploide Individuen herzustellen und erst recht, solche weiter zu züchten.

Muller hat darauf hingewiesen, daß die Erhaltung triploider und tetraploider Stämme durch den Geschlechtsbestimmungsmodus der Gonochoristen verunmöglicht wird. Möglicherweise wird es leichter sein, solche Rassen aus evertebraten Zwittern, z. B. Pulmonaten, zu züchten.

Die Methode der Bastardierung quantitativer Mutanten.

Wenn die Polyploidie Experimente eindeutig zeigen, daß die Gene bestimmte quantitative Werte haben, so ist zu erwarten, daß diese Werte auch variabel sind. Wahrscheinlich sind manche Serien multipler Allelomorphs (wie etwa die white- und die bar-Serien in Drosophila) lediglich quantitative Mutationen eines Erbfaktors (*Goldschmidt* und *Morgan-Sturtevant* sind jedoch diesbezüglich noch verschiedener Meinung). Quantitative Differenzen zeigen sich häufig in Kreuzungen geographischer Rassen oder Varietäten. Zur Demonstration schrittweiser Abänderungen besonders geeignet sind Fälle, in denen die Differenzierung während einer ersten Entwicklungsperiode im Sinne der einen Elternform erfolgt, um dann später in die andere umzuschlagen. Die zeitliche Lage des „Drehpunktes" (*Goldschmidt*) kann dann als Ausdruck der relativen Faktorenquantitäten gelten. So prüfte *Goldschmidt* z. B. eine Anzahl geographischer Varietäten des Schwimmspinners (Lymantria dispar) bezüglich ihrer Raupenfärbung. Ein extrem heller Typus wurde in Südjapan gefunden, während die Raupen der europäischen Lokalformen (Berlin) stets sehr dunkel sind. Für diese extremen sowohl als auch für verschiedene dazwischenstehende Lokalrassen wurde gezeigt, daß dieser Raupencharakter durch ein einfach mendelndes Merkmalpaar bestimmt wird. Die F_1 aus Kreuzungen folgt nun stets zuerst dem helleren Typus, verdunkelt sich jedoch mit jeder Häutung immer mehr, bis der dunklere Typus mehr oder weniger erreicht ist. Die genaue Analyse der zeitlichen Bedingungen dieser Verdunklung in den Hybriden läßt erkennen, daß der Effekt auf einer Summation quantitativer Wirkungen der beiden elterlichen Gene beruht.

Ein weiteres Beispiel einer quantitativen Analyse ist oben bereits dargestellt worden. Es handelte sich dort um die Demonstration der verschiedenen Werte eines Hermaphrodismus bewirkenden Faktors *f* in verschiedenen Lokalrassen des Grasfrosches. (Tab. 8.)

Bei quantitativen Studien macht sich in stärkstem Maße die Schwierigkeit geltend, welche jeder Vererbungsanalyse entgegensteht: das Verfahren ist ein indirektes. Der Forscher erarbeitet die charakteristischen Züge des Gens aus der phänotypischen Manifestation desselben. Es ist aber durchaus wahrscheinlich, daß in

manchen Fällen quantitative Unterschiede im Gen den Phänotypus qualitativ verändern, und noch häufiger muß es vorkommen, daß zwischen den Abänderungen im Gen und im Phänotypus keine direkte Proportionalität besteht. Bis zur Auffindung besserer Methoden wird deshalb die quantitative Analyse von Erbfaktoren auf eine relativ geringe Zahl besonders günstiger Fälle beschränkt bleiben müssen.

G. Die Mutation der Gene.

Wenn wir als Mutation jegliche Abänderung eines Gens definieren, so ist es klar, daß der Mutationsvorgang eine der Grundlagen der Stammesentwicklung sein muß — wahrscheinlich sogar die wichtigste. Damit ist jedoch nicht gesagt, daß gerade diejenigen Typen von Mutationen, die bisher von experimentellen Vererbungsforschern am eingehendsten studiert worden sind, an der Phylogenese den wichtigsten Anteil haben. Wir wissen jetzt, daß die Mehrzahl der Mutationen in Drosophila Lethalfaktoren sind (*Muller*) und eine weitere beträchtliche Fraktion setzt nachgewiesenermaßen die Lebensdauer beträchtlich herab (*Pearl*). Aber auch von der Lebenstüchtigkeit abgesehen, sind die große Mehrheit deutlich als Verlustmutanten charakterisiert. Trotz all dieser im Grunde antievolutionistischen Eigenschaften sind diese Mutationen von großer Bedeutung für die Begründung einer wissenschaftlichen Erforschung des Artbildungsproblems. Nur durch ihre systematische Bearbeitung konnte in den letzten Jahren und Dezennien ein erster Einblick gewonnen werden in den strukturellen Aufbau der chromosomalen Erbsubstanz und in die Möglichkeiten ihrer experimentellen Abänderung.

Muller und *Morgan* haben versucht, mit Hilfe der Mutationen die Größenordnung des Gens näherungsweise zu bestimmen. Einmal gehen sie aus von der folgenden Überlegung. Angenommen, ein Sack enthalte eine unbekannte Zahl von Kugeln, und es werden tausendmal je eine herausgenommen, markiert und wiederum zurückgelegt. Dann ist es klar, daß es um so häufiger vorkommen wird, daß die gleiche Kugel zweimal oder öfter gezogen wird, je geringer die Gesamtzahl ist. Aus der relativen Häufigkeit der Wiederholungen kann deshalb die Gesamtzahl der im Sack enthaltenen Kugeln berechnet werden. Nun sind bei Drosophila die meisten Mutationen ebenfalls nur ein einziges Mal beobachtet worden. Aber einige sind auch zweimal, dreimal usw. unabhängig voneinander aufgetreten. Auf Grund dieser Wiederholungen ist dann analog die Gesamtzahl der Gene mit etwa 2000 berechnet worden. Es sei hier jedoch beigefügt, daß die biologische Grundlage dieser Berechnung zum wenigsten unsicher ist. Denn es steht

jetzt fest, daß die Gene verschieden starke Neigung haben, zu mutieren. Schon 1923 berechnete *Muller* für verschiedene Gene die mittlere Lebensdauer mit „wenigen" bis zu 2000 Jahren. — Nun läßt sich ja leicht das Gesamtvolumen der metaphasischen Chromosomen bestimmen, und aus $\frac{\text{Gesamtvolumen}}{2000}$ ergibt sich für das Gen eine mittlere Größe von 0·0001 μ^3. Kugelgestalt für ein solches Gen angenommen, würde sein Durchmesser $^{60}/_{1000}\,\mu$ betragen. Wenn das Volumen des Chromatins statt aus den Chromosomen aus dem Kopfstück des Spermiums bestimmt wird, so berechnet sich der entsprechende Durchmesser mit $^{77}/_{1000}\,\mu$.

Methodisch interessanter ist vielleicht *Morgans* zweiter Versuch der Größenbestimmung. Das zweite Chromosom hat eine totale Länge von 108 Austauscheinheiten. Nun darf wohl angenommen werden, daß wir in einigen Fällen Mutationen von direkt nebeneinanderliegenden Genen kennen. Sie müssen natürlich minimale Austauschwerte haben und sich durch solche auch zu erkennen geben. Dieser Minimalwert scheint nun bei etwa 0·2% zu liegen. Die Gesamtzahl der Gene im zweiten Chromosom würde demnach 5·108 = 540 betragen. Bezogen auf das Volumen des Chromosoms im Synapsisstadium berechnet *Morgan* diesmal einen Durchmesser von $^{20}/_{1000}\,\mu$. Vermehrte und verfeinerte Beobachtungen über Mutation und Austausch werden es gewiß ermöglichen, diese Bestimmungen wesentlich zu verbessern.

Während langer Zeit waren die Versuche, experimentell Mutationen hervorzurufen, vollkommen erfolglos. Neuerdings ist es dann *Muller* (1926) gelungen, zu zeigen, daß die Temperatur wenigstens insofern einen Einfluß hat, als ihr Steigen die Frequenz der Mutationsbildung in etwa demselben Maße erhöht, wie chemische Reaktionen beschleunigt werden.

Bagg und *Little* (1923, 1924) haben als erste über von Röntgenstrahlen ausgelöste Genmutationen berichtet. Mäuse (Männchen sowohl als Weibchen) wurden mit unfiltrierten Röntgenstrahlen behandelt. Daraufhin traten in der dritten Nachkommengeneration Mißbildungen der Augen, des Kopfes, der Extremitäten und der Behaarung auf, welche sich in der Weiterzucht als vollkommen erblich erwiesen. Da die beiden ersten Generationen normal waren und eine der Abnormitäten auch in einer Kontrollzucht auftrat, so kann die Zurückführung dieser Mutationen auf die Bestrahlung nicht als vollkommen gesichert gelten.

Unterdessen hat nun aber *Muller* (1927) in Experimenten mit Drosophila demonstriert, daß in der Tat mit Röntgenstrahlen Genmutationen in fast beliebiger Zahl produziert werden können.

Meistens wurden die Spermien mit beträchtlichen Dosen bestrahlt. Eine Steigerung der Mutationsfrequenz bis zu 15.000% wurde erreicht. Die Mehrzahl der so erzeugten Mutationen sind letal, andere betreffen morphologische Charaktere. Im letzteren Falle sind die Abweichungen vom Normaltypus meist geringfügig — darin gleicht der experimentelle Mutationsprozeß dem normalen — doch erscheinen auch die stärkeren Grade. Die letzteren stimmen oft überein mit bereits bekannten Drosophilamutationen, wie z. B. white eye, miniature wing, forked bristles usw. Doch sind bereits auch einige charakteristische neue Mutationen festgestellt worden, wie splotched wing oder sex-combless. Es scheint, daß die Auslösung der Mutation nicht auf ein bestimmtes Stadium beschränkt sei, denn *Muller* erhielt Mutationen auch nach Bestrahlung unbefruchteter Weibchen und zwar nachweislich aus Eiern, die auf dem Ovocyten- o d e r auf dem Ovogonienstadium behandelt worden waren.

Genauere Angaben über die Technik und speziell die Dosierungen dieser Versuche sind in den bisher erschienenen kurzen Mitteilungen *Muller*s noch nicht enthalten.

Es ist heute noch nicht möglich abzuschätzen, wieweit die hier eröffneten neuen Methoden führen werden. Von großer Bedeutung ist aber allein schon der Nachweis, daß der Mutationsprozeß überhaupt durch äußere Faktoren beeinflußt werden kann. Es ist wohl möglich, daß, wie *Muller* hofft, das Gen wird zerlegt werden können und daß dabei seine feinere Struktur erkennbar werden wird. Arbeiten mit Drosophila sind auf diesem Gebiete besonders aussichtsreich, weil wir für diese Spezies bereits detaillierte Chromosomenkarten haben, also jede Veränderung besonders leicht erkennen und lokalisieren können. Das ist wichtig, weil durch Bestrahlung nicht nur Mutationen ausgelöst werden, sondern auch die Austauschwerte verändert erscheinen und Chromosomen überhaupt ganz eigentlich zersplittert werden können, worauf einzelne Teile derselben sich manchmal in umgekehrter Ordnung wiederum vereinigen (R e v e r s i o n), eliminiert werden (D e f i c i e n c y) oder sogar an andere Chromosomen sich anhängen (D u p l i k a t i o n und T r a n s l o k a t i o n). Ferner kann das Auseinanderweichen einzelner Chromosomenpaare in der Reduktionsteilung verhindert werden (Non-disjunction). Solche Vorgänge können bei genetisch weniger analysierten Spezies von Genmutationen oft kaum unterschieden werden. Vor den meist hermaphroditischen Pflanzen bietet diese Spezies auch den Vorteil besonders leichter Analyse aller recessiven geschlechtsgebundenen Charaktere. Recessive Letalfaktoren in einem X-Chromosom eines F_1-Weibchens geben sich in F_2 dadurch zu erkennen, daß nur die halbe Zahl der erwarteten Männchen erscheint.

H. Vererbungscytologie.

Mikroskopische Chromosomenstudien bilden die wichtigste Hilfswissenschaft der Vererbungsforschung mendelistischer Richtung. Es ist ja klar, daß die Chromosomen mit den Vererbungserscheinungen wenigstens soweit in Übereinstimmung stehen müssen, daß sie Raum für die Gene bieten. Ferner müssen sie sich während der Gametenbildung und bei der Befruchtung so verhalten, daß der Vererbungsmechanismus nie im leeren Raum schwebt. Manche Ausnahmserscheinungen im Vererbungsgang müssen sich parallel als Besonderheiten in der Übertragung von Chromosomen oder Chromosomenteilen durch die Generationsfolgen erkennen lassen. Da die Gene einzeln von ultravisibler Größe sind, ist es jedoch ganz ausgeschlossen, daß die Vererbungscytologie je allen Details der Faktorenanalyse folgen könne.

Die Methodik ist von der der allgemeinen Zytologie nicht verschieden, weshalb auf die entsprechenden Abschnitte dieses Handbuches verwiesen sei.

III. Die Analyse der plasmatischen Vererbung.

Unsere Kenntnisse über plasmatische Vererbung sind sehr spärliche. Durch die botanische Forschung sind jedoch wenigstens zwei Typen derselben aufgefunden worden.

Correns (1909) hat an weißbunten Mirabilissippen Vererbungsverhältnisse festgestellt, die vom Mendeltypus abweichen und offenbar darauf beruhen, daß die Chlorophyllkörner ausschließlich durch die Eier von Generation zu Generation übertragen werden. Da es sich hier um die Übermittlung von Plasmaeinschlüssen eines bestimmten Typus handelt, so kann man in diesem Falle von einem Plastidentypus der Vererbung sprechen. Ihm ließe sich vielleicht die Art der Symbiontenübertragung in manchen tierischen Spezies vergleichen.

Der Typus der plasmatischen Vererbung im engeren Sinne ist dann neuerdings durch *v. Wettstein* bei der Moosgruppe der Funariaceen einer sorgfältigen Analyse unterworfen worden. Es zeigt sich, daß das Plasma im allgemeinen um so differenter ist, je ferner sich die Formen in systematischer Hinsicht stehen. Die plasmatischen Unterschiede manifestieren sich deshalb vorwiegend in Artkreuzungen, und zwar am sinnfälligsten darin, daß die F_1-Generationen reziproker Kreuzungen ungleich, in der Regel matroklin sind. Auf *v. Wettsteins* interessante Arbeiten im Detail einzugehen ist hier nicht der Ort. Wir beschränken uns darauf festzustellen, daß Erbfaktoren des Plasmas systematisch analysiert werden konnten. Wie im Kern das Gen, so erscheint im Zellplasma das Plasmon lokalisiert.

Es gibt nun zahlreiche Anhaltspunkte dafür, daß solche Plasmone auch im Tierreich ihre Rolle spielen und der Analyse zugänglich gemacht werden könnten. Es liegen auch hier viele Angaben über Matroklinie von Artbastarden vor, worunter als bekannteste die Pferd-Esel-Kreuzungen zählen. Ebenso bedeutsam sind die Ergebnisse der Merogonieversuche. Wenn entkernte Eier oder Eifragmente durch artfremde Spermien befruchtet werden, so entwickeln sie sich nur bis zu einem gewissen Stadium, und zwar ergeben Untersuchungen an Echinodermen und Amphibien übereinstimmend, daß die Entwicklung um so weiter geht, je näher verwandt die Ei- und Spermaspezies sind. Über die Natur der hier beteiligten plasmatischen Faktoren kann man heute noch kaum auch nur Vermutungen äußern. Artbastardierungen und Merogonieversuche scheinen am ehesten berufen, dieses Problem einer Lösung entgegenzuführen.

Schließlich kommt bei Tieren noch ein Typus matrokliner Vererbung relativ häufig zur Beobachtung, der wohl als p s e u d o p l a s m a t i s c h e V e r e r b u n g bezeichnet werden könnte. Es ist klar, daß die Struktur des reifen und zur Befruchtung bereiten Eies durch die genetische Konstitution der Mutter bestimmt wird. Darum müssen notwendigerweise alle Charaktere, die durch die „Eistruktur" bestimmt werden — sie werden sich namentlich während der ersten Embryonalperioden manifestieren — m ü t t e r l i c h e s G e p r ä g e zeigen. So ist wiederum bei Spezieskreuzungen stets festgestellt worden, daß der Rhythmus der Furchungsteilungen von der Eispezies allein bestimmt wird. *Driesch*, *Boveri*, *Peter* u. a. haben gezeigt, daß Echinidenbastarde sogar bis über die Gastrulation hinaus matrokline Züge erkennen lassen. Von großem Interesse ist der Vererbungsmodus links- und rechtsgewundener Schnecken (Partula, Limnaea) namentlich im Lichte der Analyse *Sturtevants*, die klar erkennen läßt, daß das Individuum nicht gemäß seiner persönlichen Genkonstitution, sondern entsprechend der seiner Mutter erscheint. Gerade dieser Fall zeigt, daß Matroklinie auch auf Mendelvererbung beruhen kann, mit der Besonderheit jedoch, daß die Determination des Phänotypus schon vor der Befruchtung und vor der Reduktionsteilung im Ei, somit konform der mütterlichen Genkonstitution erfolgt.

SCHLUSS.

Die Vererbungsforschung ist in mancher Hinsicht eine Sammelwissenschaft. Außer mit den speziellen Erscheinungen der Übertragung der Eigenschaften von Generation zu Generation beschäftigt sie sich mit zahlreichen praktischen und theoretischen Grundproblemen der biologischen Schwesterdisziplinen. Dieser

Zustand spiegelt sich notwendigerweise in ihrer Technik wider. Wenige Arbeitsmethoden sind für die Vererbungswissenschaft spezifisch. Der Genetiker sollte gleichzeitig Chemiker, Physiker, Biometriker, Physiologe, Cytologe, Embryologe, Systematiker und Tiergeograph sein, um verschiedenste Methoden sinngemäß auf Vererbungsprobleme anwenden zu können.

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INTEGUMENTARY PIGMENTATION IN THE FROG, RANA PIPIENS, DURING METAMORPHOSIS, WITH ESPECIAL REFERENCE TO TAIL-SKIN HISTOLYSIS

(Two plates and one figure)

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INVESTIGATIONS upon the specificity of integument in amphibians have been confined to a comparatively recent date. Considerable experimental work has been reported within the last few years, the greater part of which has been upon the salamander. In this regard the findings of Weigl (1913) and Uhlenhuth (1917) are of interest. The former found that homoplastic transplants of salamander (*Salamandra maculosa*) larvae integument retained their specific pigmentary characteristics through metamorphosis. Uhlenhuth, working with *Amblystoma punctatum*, made similar observations and concluded that two factors were responsible for pigmentation: one within the skin itself, and one which was within the blood stream and which he calls a "metamorphosis factor." Winkler (1910) made homoplastic, integumentary transplants on *Hyla* and found that belly and back skin retained their pigmentary characters indefinitely.

A more recent work in this connection has been reported by Cole (1922), working with *Rana clamitans* and *R. catesbeiana* larvae. He noted that tail, back, and belly skin transplanted to any region of the body maintained its individual pigmentary characteristics indefinitely during the larval life of the animal.

It seemed worth while that a study be made of pigmentary reactions in integumentary grafts as they occur during metamorphosis. Series of operations were accordingly planned which involved the reciprocal transplantation of larval skin grafts from areas known to differ widely in pigmentation pattern following metamorphosis. In this way light could be thrown on the possible specificity or nonspecificity of integumentary pigmentation. If the integument is nonspecific in this respect, then we should expect transplanted skin grafts to assume the pigmentation, during metamorphosis, typical

of the region to which they had been transplanted. Conversely, if the integument is specific, we should expect skin grafts to develop the same pattern and type of pigmentation during metamorphosis in the foreign location as they would have developed if not transplanted.

Another question might well be raised regarding the mechanism of integumentary histolysis in metamorphosing tadpoles. Several theories have been formulated in an attempt to explain the factors responsible for inducing tail atrophy. Among the most important is that of Barfurth (1887), who maintained that the growth of the pygostyle initiates the process of tail atrophy by occluding the blood vessels at the base of the tail. Bataillon (1891) likewise thought that the rapidly growing pygostyle caused a change in the distribution of the blood supply to the tail. Dr. O. M. Helff of this laboratory, in unpublished investigations, has recently removed the pygostyle from normal tadpoles and finds no appreciable difference in the rate at which the tail atrophies during metamorphosis. In no case was normal atrophy delayed. Morse (1918) suggested that the atrophy is probably induced by a condition of acidosis in the muscle tissue of the tail, the causation of which was problematic.

Interesting as these investigations are, relatively few observations have been made regarding the mechanism of integumentary histolysis. Helff (1926) produced integumentary histolysis in tadpoles by transplanting atrophying gill and tail muscle beneath opercular skin grafts. This author suggested the possibility that all atrophying or degenerating tissue in metamorphosing larvae may be capable of initiating integumentary histolysis when in contact with the integument.

In this connection it seemed profitable that a study be made regarding the specificity of histolytic reactions in tail and back skin grafts as they occur during metamorphosis. Series of transplantation experiments were planned which involved an interchange of larval skin grafts from the tail and back. If tail skin is specific as regards histolysis, we should expect it to undergo disintegration during metamorphosis, when transplanted to a foreign location. Should the tail skin be non-specific, however, in this respect, and subject to histolysis because of its association with other structures of the tail,

we should not expect it to undergo disintegration during metamorphosis when transplanted to foreign locations.

The present work was carried out at the Iowa Lakeside Laboratory during the summer of 1928. I am indebted to Dr. O. M. Helff for suggesting the problem and for his valuable suggestions and criticism during the course of the work.

MATERIAL AND METHODS

The species of frog larvae used in this investigation was that of *Rana pipiens*. The larvae were collected from a small pond near Spirit Lake, Iowa, during the months of June and July. The animals were taken to the laboratory, placed in large battery jars, and fed daily with fresh *Spirogyra*. From this common stock, normal tadpoles of a uniform size were selected for the various experiments. The individuals selected for use ranged from 60 to 100 mm., body length, with hind limbs varying between 4 and 15 mm. The animals used were normal tadpoles and showed no signs of metamorphosis for at least 2 weeks following the operations.

All experiments necessitated the use of an anesthetic which would render the animal inactive for some time. For this purpose a 0.05 per cent aqueous solution of chloretone was employed. The time required for anesthetization by this method varied from 5 to 10 minutes, depending somewhat upon the size of the animal and the temperature of the chloretone solution. The animals were allowed to remain in the weak chloretone solution for a few minutes after they failed to respond to any mechanical stimuli. Animals so anesthetized remained inactive for at least 2 hours, thus giving adequate time for operative work and postoperative adhesion of grafts and cut edges.

The usual method of skin transplantation was employed. Following the anesthetization, the animal was placed upon a damp cloth under a binocular microscope. An incision was first made, by means of a fine pair of dissecting scissors, around the area of skin to be transplanted. The skin was then stripped from the underlying tissue and transplanted to another region of the body.

With the exception of Series C, autoplastic transplants were made consisting of an interchange of skin grafts from two regions of

the same animal. Figure A illustrates the regions from which the transplants were taken and the areas to which they were grafted. Following the operation, the animals were placed in a Petri dish, partially filled with water, in such a way that the operated areas were exposed to the air. One-half hour of such exposure was usually adequate to allow sufficient adhering of the grafts so that the animals could be immersed completely in water. Daily observations were then made of each individual in respect to the healing process, pigmentary changes, and the progress of metamorphosis. A complete record was maintained for each animal.

RESULTS

SPECIFICITY OF INTEGUMENTARY PIGMENTATION DURING METAMORPHOSIS

1. *Types of skin transplantations.*—Series A consisted of autoplastic skin transplants in which skin from the back was interchanged with a similar graft from the right side (Fig. A, *B*, *S*). Care was

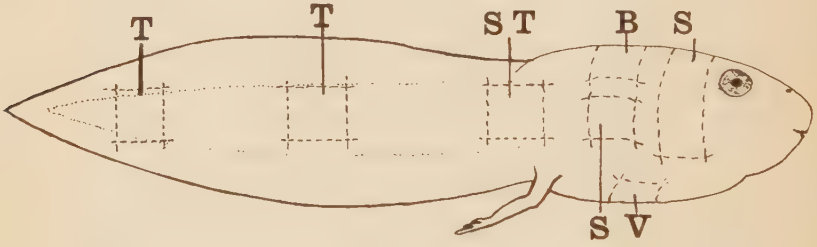


FIG. A

taken to obtain skin grafts from the regions known to develop definite pigment patterns during metamorphosis. Furthermore, the grafts were so placed that their original anteroposterior orientation was not changed. Eighteen cases were obtained, ten of which were permitted to metamorphose completely, the remaining eight being fixed for histological study at various stages of metamorphosis. A second series (Series B) consisted of removing a section of skin about 1 cm. wide, extending from the mid-dorsal to the mid-ventral line. This graft was then rotated 180°, so that the original dorsal or back portion was in contact with the belly and vice versa (Fig. A, *S*, *B*).

Of this type of operation, twelve animals underwent complete metamorphosis, while six were fixed at different stages for histological study.

In consequence of several difficulties encountered in making reciprocal, autoplastic transplants from back to belly, a third type of operation (Series C), consisting of homoplastic skin transplants from the foregoing regions, was carried out (Fig. A, *B*, *V*). The operation consisted of removing a section of skin from the back of an animal and replacing it with a similar graft taken from the belly of another, and in turn transplanting the section taken from the back of the former to the belly of the latter. Eleven animals in this group were allowed to metamorphose, while several were utilized for histological study.

The animals used for the foregoing series were normal tadpoles in every respect, ranging from 70 to 80 mm. in length. The time required for the grafts to heal varied somewhat, depending on the size of the graft, large grafts requiring a slightly longer period, due undoubtedly to the larger wound-area present. Normally 8–10 days were found sufficient for the process to take place. In the course of the healing process it was noted that the grafts appeared slightly darkened for at least 2 days following transplantation. At the end of this period, however, the grafts quickly regain their normal healthy color. However, it was noted that a slightly darkened area persisted around the border of the graft. Upon examination this was found to be due to several types of pigment cells which had been carried into that region along with the migration of epithelial cells which cover it. This darkened area remained for a considerable period of time, often long after metamorphosis.

2. *The development of characteristic pigment patterns in grafts.*— The foreign locations of the various skin grafts in no case effected their pigmentation pattern during metamorphosis (Plate I, Figs. 1, 2, 3, 4, 5). Of the forty-one cases obtained, all retained their specific pigmentary characteristics. Skin transplanted from back to side, or side to back, as in Series A, developed normal spotting characteristic of the region from which they had been taken. As far as could be determined, the number of spots, their intensity of color, and orientation were identical with those of normal integument. Skin trans-

PLATE I

FIG. 1.—Side graft transplanted to back. *G*, graft; *B*, back skin; *P*, pigment spot, showing migration of pigment spot into graft.

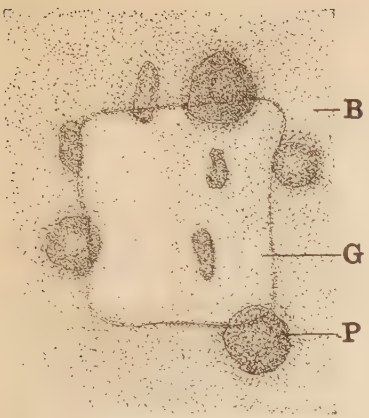
FIG. 2.—Belly graft on back. *G*, graft; *B*, back skin; *P*, pigment spot, showing failure of spots to migrate into graft.

FIG. 3.—Back graft on belly. *G*, graft; *V*, belly skin; *P*, pigment spot. No pigment migration from graft.

FIG. 4.—Back graft to side. *G*, graft; *S*, side skin; *P*, pigment spot. Very slight pigment migration.

FIG. 5.—Back-skin graft on atrophying tail. *G*, back-skin graft; *T*, tail integument; *P*, pigment spots. Showing normal development of back skin on atrophying tail.

PLATE I



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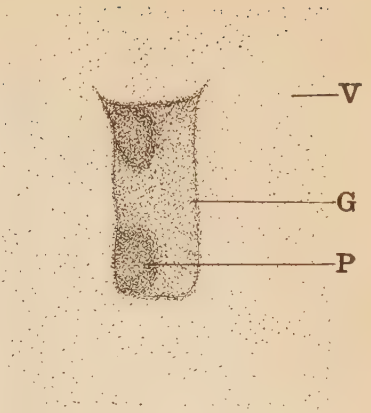

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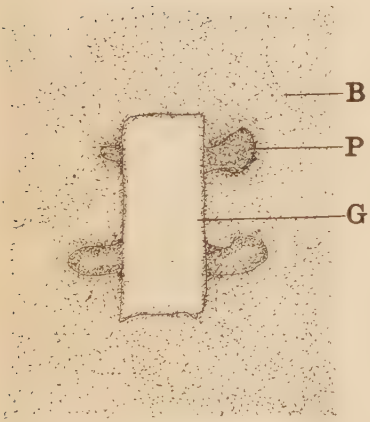

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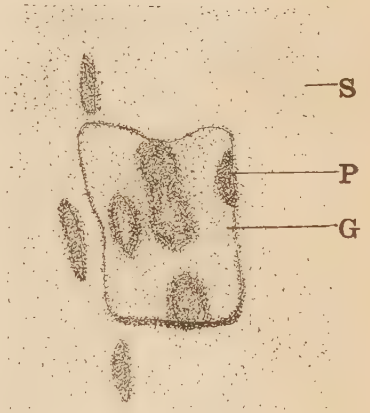

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planted from the back to belly produced its normal spotting in the new position.

Histological examination of the various grafts gave no evidence of a marked decrease or increase in the amount of pigment. A slight variation was noted, however, in Series A, and to a lesser extent in the other series. This consisted of a general tendency of the pigment composing the spots of the normal host skin to migrate slightly into the graft. Plate I, figure 1, represents a graft from the side transplanted to the back, in which this occurred. This was a frequent occurrence in this type of graft and was found to be true in at least 75 per cent of the cases. It was only in this type of graft, however, that such pigmentary migration was observed. In the grafts taken from the back and transplanted to the side, for example, no such migration occurred from either the graft or host skin (Plate I. Fig. 4). The same holds true for back to belly and for belly to back grafts (Plate I, Figs. 3, 2). Upon close examination it was found that such migration took place in the epithelial layer of the skin only, and was therefore quite superficial. By means of a sharp knife it was possible to peel off the layer of epithelial cells from the underlying tissue of the integument, and with it all signs of the newly pigmented area. From the foregoing results, it would appear, therefore, that the factors responsible for the development of pigmentation and spotting during metamorphosis are localized within the integument itself and that the latter is therefore specific in this respect.

MECHANISM OF TAIL-SKIN HISTOLYSIS DURING METAMORPHOSIS

Series D consisted of thirty-five cases of autoplastic skin transplantations which were of two types. The first type involved an interchange of integument from the back with a similar section from the tail (Fig. A, *B*, *T*). The second type consisted of an interchange of a section of skin from the junction of the tail and body with a similar graft from the back (Fig. A, *ST*, *B*). Of this series, twenty-one animals survived metamorphosis, the rest being killed for histological study at various metamorphic stages. Sixteen of these were of the first type and five of the second type of transplantation. In all cases the transplantations were made from 2 to 4 weeks prior to

the onset of metamorphosis, which allowed sufficient time for complete healing of the transplant. The time required for healing was the same as that noted in Series A, B, and C, the grafts regaining their normal appearance in about 1 week, indicating that normal circulation had again been restored to the grafted tissue. The grafts invariably retained their normal pigmentation up to the stage of metamorphosis at which integumentary spotting normally occurs.

The results of Series D emphasized the local specificity of integument as regards pigmentation during metamorphosis, as was shown in Series A, B, and C. Back skin transplanted to regions on the tail developed the normal pigmentary characteristics typical of the region from which it had been taken. Instead of histolyzing and being absorbed in consequence of its contact with atrophying tissue during metamorphosis, as would be expected, it invariably developed the normal pigment pattern characteristic of back skin. The normal greenish coloration and dark pigmented spots appeared with the same intensity as that typical of normal back skin (Plate I, Fig. 5). However, with the resorption of the tail and its integument, the grafts migrated from their original transplanted position to one adjacent to the tip of the urostyle, where they remained indefinitely. Grafts transplanted from the back to the posterior end of the tail underwent the same pigmentary changes during metamorphosis as those transplanted nearer the anterior region. The pigmentary changes of such grafts were found to take place simultaneously with the spotting of the back.

Histological examination of the grafts showed no signs of cellular histolysis, the integument having the appearance of normal back skin in every respect. Plate II, Figure 6, represents a typical section made from such a graft removed at a stage when the tail had been reduced to 1 cm. in length. The *stratum compactum* layer of the host skin, it will be noted, has undergone an almost complete degeneration, the epithelial cells being crowded together somewhat while the pigment layer beneath has become more compact. The graft, however, appears normal in every respect, and a very definite junction line appeared between it and the histolyzing skin of the tail.

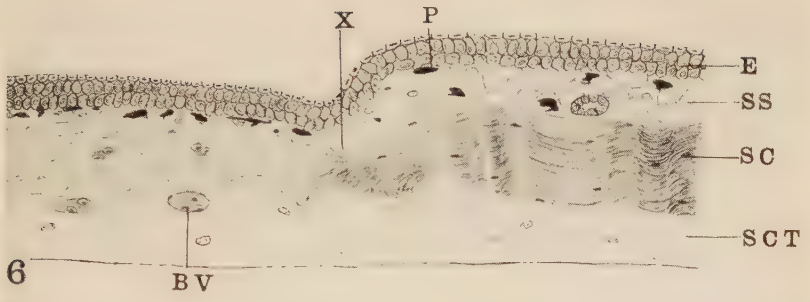
The reciprocal graft, however, in which tail skin was transplanted to the back, underwent degeneration, during metamorphosis, and

PLATE II

FIG. 6.—Section through back graft transplanted on tail and of adjacent atrophying tail integument. *E*, epidermis; *SS*, *stratum spongiosum; SC*, *stratum compactum; SCT*, subcutaneous connective tissue; *P*, pigment masses; *BV*, blood vessels; *X*, junction of back graft and atrophying tail skin. *Stratum compactum* dissociated, crowding together of epithelial cells and pigment masses, of tail integument. Graft normal in every respect.

FIG. 7.—Section through tail graft transplanted to back. Note *stratum compactum*, pigment masses, and epithelial cells. Showing normal histolysis of tail integument when transplanted to the back. *X*, junction of tail-skin graft and back skin.

FIG. 8.—Graft obtained from junction of tail and side. *S*, side portion; *T*, tail portion; *AT*, atrophying tail. Macroscopic appearance of graft during metamorphosis. Tail portion histolyzing; side portion developing normal pigmentation.



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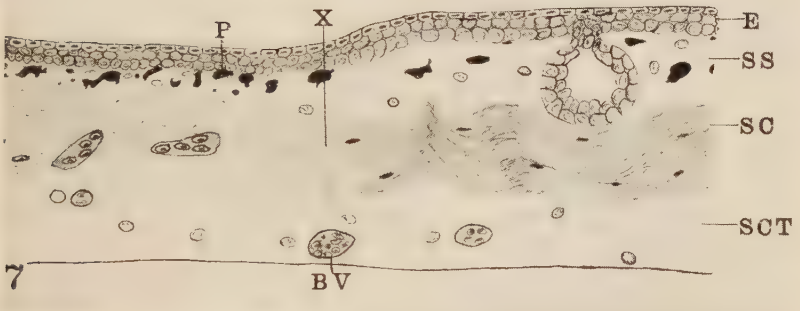

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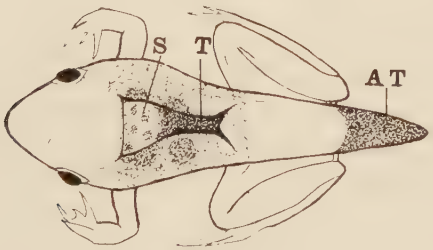

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presented all the external signs of histolysis. When transplants of tail skin were made to the back, the normal process of healing took place, as was found for other types of skin grafts. Such grafts retained their normal pigmentation and apparently acquired a normal blood supply. However, at the stage of metamorphosis when tail skin normally undergoes visible histolysis, the grafts become slightly darker in color corresponding to the darkening of the integument of the atrophying tail. As metamorphosis progressed, the grafts were resorbed, again corresponding exactly with the resorption of the integument of the tail.

Histological examination showed histolysis identical to that typical of histolyzing tail integument during the atrophy of the tail. This will be made clear by reference to Plate II, Figure 7, which depicts normally histolyzing tail skin. In this connection, it may be said, a complete study was not undertaken regarding the histological sequence of events which take place in this type of histolysis, the study being more from a macroscopical point of view. It may be said, however, that the microscopical picture of tail-skin histolysis obtained by the writer compares quite closely with those described and pictured by Helff (1926), for opercular integument.

Table II, Figure 8, represents a second type of tail-skin transplantation, in which a graft obtained from the junction of the tail and side of body, including skin from both regions, was interchanged with a back-skin graft. Similar to the foregoing results, an absorption of the tail integument occurred, during metamorphosis, while that of the side developed into normal side skin. Histological sections of such grafts were similar to those represented in Figures 6 and 7, the tail-skin portion of the graft always undergoing typical histolysis while the side-skin part remained normal in all respects.

DISCUSSION

The results of skin transplantation experiments have served to emphasize certain fundamental principles involved in the mechanisms of integumentary metamorphosis. An important point which has been frequently emphasized is that of specificity of pigmentation. The work of Uhlenhuth (1918) in this regard is of interest. He concluded that two factors are involved in the development of pigmen-

tary spots in *Amblystoma*, one factor which is contained within the skin itself, and a second, which he calls "metamorphosis factor," contained not in the skin but in the circulation. He states regarding the latter, "It may be identical with an agent like the thyroid substance." Weigl (1913), however, believed that in the salamander the metamorphosis of the skin is a process of self-differentiation.

The present work indicates quite strongly that the factors responsible for the development of pigmentation in the frog during metamorphosis are from more than one source. From the results obtained, it seems quite probable that the presence of a specific hereditary factor within the skin itself cannot be denied. In other words, the various areas of integument of the tadpole have latent within them the specific pigment cells or factors to develop, at a certain stage of metamorphosis, the pigment pattern characteristic of the adult. The development of the characteristic pigmentation pattern in integument when transplanted to foreign locations would seem to fully substantiate such a conclusion.

At the same time, however, it is logical to assume the presence of some factor, or factors, within the blood stream which is active or becomes active at a certain stage of metamorphosis. Such a factor, or factors, might well be conceived as being responsible for the rapid development of the latent pigmentation pattern present in the various areas of the integument.

The results of the present work serve to throw light on the possible specificity and mechanisms of tail-skin histolysis as it occurs during the normal atrophy of the tail during metamorphosis. It has been suggested by Barfurth (1887) and Bataillon (1891) that atrophy of the tail is induced primarily by the rapid growth of the pygostyle during metamorphosis, which serves to at least partially occlude the blood supply to the tail. This, according to Morse (1918), would be a possible explanation for the condition of acidosis which occurs in the tissues of the atrophying tail. Morse, furthermore, points out that the acidosis which is present is sufficient to bring about autolytic reactions, which in turn result in the atrophy of the tail.

Although the foregoing explanation might serve to explain the degeneration of certain tissues of the tail, particularly muscular

tissue, it cannot be accepted by the writer when applied to the histolysis of tail integument during metamorphosis. The fact that tail integument transplanted to the back undergoes normal histolysis without the presence of adjoining atrophying tissue requires some other explanation. Moreover, the fact that integument from the back transplanted to the tail never undergoes histolysis during tail atrophy, is a negation of the idea that tail skin undergoes histolysis during metamorphosis entirely because of its association with the atrophying musculature beneath. In this connection the work of Helff (1926) is of interest. The latter author induced histolysis of opercular skin grafts transplanted to the back by insertion of atrophying tail muscle beneath such grafts. Although concerned primarily with histolysis of opercular integument as it occurs during the formation of the opercular fore-limb perforations during metamorphosis, the preceding experiment led Helff to suggest the probability that histolysis of tail integument, as it normally occurs, might be due to the presence of the underlying atrophying musculature of the tail. The results of the present paper indicate clearly, however, that complete histolysis of tail skin can take place when transplanted to a foreign location and without association with other atrophying tissue of the tail.

Therefore the mechanism of tail-skin histolysis as it occurs during metamorphosis would appear to be a twofold one. The integument of the tail must be specific, hereditarily, to undergo a process of histolysis when subjected to certain influences during metamorphosis. Secondarily, the results justify the assumption that this influence must be transported through the blood stream and is liberated or becomes active in the latter at a definite stage of metamorphosis. The exact nature of this influence, although probably hormonic, is quite problematic.

SUMMARY AND CONCLUSIONS

1. Several types of larval skin-transplantation experiments were made in an attempt to determine the local specificity of frog integument when transplanted to foreign regions and allowed to metamorphose. The grafts involved skin from the back, belly, side, and

tail. Observations were made with respect to the specificity of integument for pigmentation and histolysis.

2. Forty-one cases of autoplastic and homoplastic larval skin grafts showed local specificity for pigmentation when allowed to metamorphose. All grafts invariably developed the pigment pattern characteristic of the region from which they had been removed. It is concluded that the integument of the frog is specific for pigmentation, and that the factor responsible for the development of the normal pattern is contained within the skin itself.

3. Similar transplants involving an interchange of tail skin with skin from the back suggested the specificity of tail skin toward histolysis. Tail skin, when transplanted to the back, undergoes histolysis during metamorphosis. Conversely, skin transplanted from the back to the tail undergoes normal development during metamorphosis and develops the pigmentary pattern characteristic of the region from which it was taken. It is concluded that two factors determine the histolysis of tail integument, one of which lies within the skin itself and is specific, and the second, within the blood stream, which becomes functional in inducing histolysis of tail integument at a certain stage of metamorphosis.

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